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ON THE DECOMPOSITION OF DIAZO COMPOUNDS.

VII.—ON THE REACTION OF ALCOHOLS WITH PARADIAZOBENZENESULPHONIC ACID.[1]

BY W. B. SHOBER.

This investigation was undertaken at the suggestion of Professor Remsen, and carried on under his guidance, with the object of determining the action of methyl, ethyl and propyl alcohols on paradiazobenzenesulphonic acid, at various pressures.

16 grams of the diazo compound were boiled with about 250 cc. of methyl alcohol (distilled from lime) under a pressure of 760–780 mm. of mercury. At first the alcohol assumed a yellow color, then changed to pink, and finally the liquid became dark red. The decomposition was slow, about two and a half hours being necessary to decompose all of the diazo compound. The liquid obtained in this way is very strongly acid. The alcohol was distilled off, leaving a dark syrupy residue. Repeated attempts to obtain crystals from this residue, after purification with animal charcoal, were unsuccessful. The residue was dissolved in water and boiled with animal charcoal, nearly all the color being removed by this means. It was then neutralized with barium carbonate; a slight precipitate of barium sulphate was formed which was filtered off. The sodium salt, or rather salts (for, as shown below, two reactions took place), were made by precipitating the barium as carbonate. It was found necessary to use

[1] From the author's thesis for the degree of Doctor of Philosophy. Submitted to the Board of University Studies of the Johns Hopkins University, June, 1892.

the sodium compound, as the corresponding barium salts do not react readily with phosphorus pentachloride. The sodium salts were treated with one molecule of phosphorus pentachloride. The action takes place easily without the aid of heat. When the reaction was completed the flask containing the products was heated to 120° in a sulphuric-acid bath, in order to drive off the phosphorus oxychloride. The sulphonic chlorides were washed with a large amount of water. Concentrated ammonium hydroxide was then poured over the oil and the mixture allowed to stand for some time. The amides thus obtained were boiled with animal charcoal and recrystallized several times. Two different kinds of crystals were found which were separated by mechanical means. One substance crystallized in beautiful, long, slender needles, the melting-point of which was found to be 113°. This substance is shown by the analysis given below to be methoxybenzenesulphonamide, presumably the para modification, a compound which I believe has not been described before. The other compound found also crystallizes in needles, but they are much smaller. Its method of formation and melting-point (154°) show it to be parabenzenesulphonamide. The substance melting at 113° is insoluble in cold water, easily soluble in hot water and in alcohol. Analysis shows its composition to be $C_7H_9SNO_3$. The results obtained are as follows:

I. 0.29537 gram of the substance gave 0.36758 gram barium sulphate, corresponding to 17.10 per cent. of sulphur.[1]

II. 0.12373 gram of the substance gave 0.15517 gram of barium sulphate, corresponding to 17.23 per cent. of sulphur.[1] These determinations were made by Liebig's method.

I. 0.21172 gram of the substance gave 0.34615 gram of carbon dioxide and 0.08958 gram of water.

II. 0.18255 gram of the substance gave 0.29735 gram of carbon dioxide and 0.08292 gram of water.

For $C_7H_9SNO_3$:

		Theory.	Found. I.	Found. II.
C_7	83.79	44.89	44.58	44.41
H_9	9.00	4.82	4.71	5.15
S	31.98	17.13	17.10	17.23
N	14.01	7.51	7.26	7.58
O_3	47.88	25.65		
	186.66	100.00		

[1] Messrs. Hunter and Collier kindly made these determinations for me.

The compound is, therefore, methoxybenzenesulphonamide, and since it is obtained from sulphanilic acid in which the amido and sulphonic acid groups are in the para position, it is para-methoxybenzenesulphonamide.

Besides the methoxy compound there is also formed benzenesulphonic acid. Consequently the product obtained from the decomposition of the diazo compound is a mixture. Since the amides of these two acids are well crystallized substances, an attempt was made to separate them by fractional crystallization, in order to form a definite idea as to the relative quantities formed in the decomposition. This attempt was unsuccessful, owing to the fact that both amides are about equally soluble in water, alcohol, ether, and mixtures of alcohol and water in various proportions. The following experiments were made with the object of finding a method for separating the two acids:

A quantity of the diazo compound was decomposed with methyl alcohol at a pressure of 760–780 mm. of mercury. After the reaction was completed, the excess of alcohol was distilled off, the syrupy residue diluted with water and treated with animal charcoal. A portion of this product was neutralized with barium carbonate. The solution of the barium salts was evaporated to dryness and the residue boiled with alcohol. A portion dissolved.

Analysis of the portion soluble in alcohol:

0.1713 gram of the substance gave 0.0825 gram of barium sulphate, corresponding to 28.33 per cent. of barium. The theoretical per cent. of barium in barium methoxybenzenesulphonate is 26.83, and in barium benzenesulphonate, 30.40.

Analysis of portion insoluble in alcohol:

0.12275 gram of the substance gave 0.0574 gram of barium sulphate, corresponding to 27.50 per cent. of barium. Similar experiments were made, using methyl alcohol as the solvent, with the following results:

0.15112 gram of the substance soluble in methyl alcohol gave 0.074 gram of barium sulphate, corresponding to 28.80 per cent. of barium.

0.14215 gram of the substance insoluble in methyl alcohol gave 0.07071 gram of barium sulphate, corresponding to 29.26 per cent. of barium.

It is evident from these results that the barium salts cannot be separated in this way.

Attempts to separate the two Acids by means of the Anilides.— Aniline was added slowly to the chlorides; action took place with evolution of heat. The anilides thus obtained were washed with dilute hydrochloric acid, and afterward with a *small* quantity of caustic soda. Care must be taken not to use too much of this reagent, as it dissolves the anilides. Water is used to remove the caustic soda. The anilides were then crystallized from water, or preferably, from a mixture of equal parts of water and alcohol. Two kinds of crystals were deposited, small needles and irregular shaped plates, but I was unable to obtain a satisfactory separation in this way.

The following salts of the two acids were then prepared: barium, sodium, ammonium, potassium, calcium, silver, lead, copper, and zinc.

The barium, calcium, lead, and zinc salts were prepared by neutralizing the acids with the corresponding carbonates. The sodium, ammonium, and potassium salts were made from the barium salts by precipitating the barium as carbonate: the copper[1] and silver salts by precipitating the barium salts with the corresponding sulphates. It was found impossible to separate satisfactorily any of these salts by fractional crystallization.

An attempt was then made to obtain an idea of the relative amounts of the two acids formed in the decomposition of the diazo compound with methyl alcohol, by converting the products into the barium salts, determining the percentage of barium in the mixture of the two, and upon the basis of the results thus obtained to draw a conclusion as regards the relative quantities of the different acids obtained in the decomposition. With this object in view I made the following barium determinations:

I. 0.07865 gram of the barium salts gave 0.03675 gram of barium sulphate, corresponding to 27.48 per cent. of barium.

II. 0.1174 gram of the barium salts gave 0.05485 gram of barium sulphate, corresponding to 27.47 per cent. of barium.

III. 0.13885 gram of the barium salts gave 0.0645 gram of barium sulphate, corresponding to 27.32 per cent. of barium.

Six determinations of this kind were made, but, owing to variations in the results, not due to errors of analysis, no satisfactory conclusions could be drawn. Owing to a lack of time I was unable to continue experimenting along this line, and in consequence a

[1] Freund: Ann. Chem. (Liebig) **120**, 80.

method for the separation of the products of the decomposition of the diazo compound with methyl alcohol has not been found. However, the following experiments will show conclusively the conditions favorable for the formation of the methoxy compound.

Action of Methyl Alcohol on Paradiazobenzenesulphonic Acid at a Pressure of 850 mm. of Mercury.

26 grams of the diazo compound and 520 cc. of methyl alcohol, distilled from lime, were placed in the pressure-flask. Heat was applied until a pressure of 850 mm. (the highest pressure that could be obtained with the apparatus used) was indicated by the manometer. The decomposition was complete in two and a half hours. The alcohol was then distilled off and the dark red liquid residue treated with animal charcoal. It was then converted into the amides as above described. Only a partial separation of the amides could be effected, but it was sufficiently complete to show the presence of the paramethoxybenzenesulphonamide in larger quantities than the benzenesulphonamide.

Action of Methyl Alcohol on Paradiazobenzenesulphonic Acid at a Pressure of 360 mm.

26 grams of the diazo compound and 520 cc. methyl alcohol were placed in the flask and heated until the pressure indicated was 340–360 mm. The experiment was carried out as above. The decomposition was much slower, the time required being a little more than five hours. The products of decomposition were transformed into the amides. The quantities of the methoxy- and hydrogen-amides were about equal.

Action of Methyl Alcohol on Paradiazobenzenesulphonic Acid at Ordinary Pressure.

10 grams of the diazo compound and 200 cc. methyl alcohol were placed in a flask to which was attached an inverted condenser. The flask was heated on a water-bath. The action took place slowly and was complete in three hours. The products of decomposition were treated as above. Two amides were obtained, the hydrogen-amide being present in larger quantity than the methoxy-amide.

Action of Methyl Alcohol on Paradiazobenzenesulphonic Acid at Diminished Pressure.

The apparatus used in this experiment was devised by Mr. R. M. Parks, of this laboratory, and used by him in his study of the action of methyl alcohol on paradiazoorthotoluenesulphonic acid.[1] It is very simple and answers the purpose for which it is intended, admirably.

10 grams of the diazo compound and 200 cc. methyl alcohol were placed in the flask, which was heated on a water-bath. The experiment was conducted in the ordinary way, the pressure being 450 mm. less than the atmospheric pressure. Eight hours were required to effect complete decomposition. The product was converted into the amide. But *one* amide was formed, the melting-point of which (154°) was that of benzenesulphonamide, showing that the hydrogen reaction only took place.

These experiments led to the conclusion that if a sufficiently high pressure could be obtained, the decomposition would result in the formation of the methoxy compound only. With this object in view I made the next experiment.

Action of Methyl Alcohol on Paradiazobenzenesulphonic Acid at a Pressure of 30 Atmospheres.

1 gram of the diazo compound and 10 cc. methyl alcohol were placed in a hard-glass tube, which was then sealed. It was heated for two hours at a temperature of 160° in a Carius furnace. The reaction took place readily, a dark red liquid being formed. The pressure in the tube, calculated from the vapor tension of methyl alcohol at 160°, was at least 30 atmospheres The decomposition probably takes place at a pressure much lower than this. The product of the decomposition was converted into the amide, which was found to be the *methoxy* amide. This was the *only* amide formed.

This experiment completes the chain of evidence showing that low pressure favors the hydrogen reaction and high pressure the methoxy reaction. This is in accordance with the results obtained in previous investigations carried on in this laboratory. Having arrived at these results with methyl alcohol, the idea of investigating the action of ethyl and propyl alcohols under the same conditions naturally presented itself.

[1] This Journal **15**, 320.

Action of Ethyl Alcohol on Paradiazobenzenesulphonic Acid at Ordinary Pressure.

For this and the succeeding experiments with this alcohol, ethyl alcohol of 95 per cent., distilled from lime, was used. 10 grams of the diazo compound and 200 cc. of ethyl alcohol were placed in a balloon flask, to which was attached an inverted condenser. Upon applying heat the alcohol assumed a yellow color, which gradually passed through pink to a deep red. The odor of aldehyde was plainly perceptible during the course of the experiment. About two hours were required for complete decomposition. The deep red liquid was then freed from the alcohol, which remained unacted upon, and after purification with animal charcoal was converted into the amide. But *one* amide was found, and its crystal form and melting-point showed it to be benzenesulphonamide.

Action of Ethyl Alcohol on Paradiazobenzenesulphonic Acid at a Pressure of 350 *mm.*

10 grams of the diazo compound and 200 cc. ethyl alcohol were placed in the pressure-flask. Heat was applied until a pressure of 350 mm. was indicated by the manometer. The pressure was held at this point until the decomposition was complete, one and a half hours being required for this purpose. The product of decomposition was treated as before and converted into the amide. Only *one* amide was formed, and this was benzenesulphonamide.

Action of Ethyl Alcohol on Paradiazobenzenesulphonic Acid at a Pressure of 840 *mm.*

10 grams of the diazo compound and 200 cc. ethyl alcohol were placed in the pressure-flask. The decomposition took place at a pressure of 820–840 mm., about seventy minutes being required for complete decomposition. The product was converted into the amide as above. Only *one* amide was found. This was benzenesulphonamide.

These experiments show that when ethyl alcohol acts upon diazobenzenesulphonic acid under the varying conditions of pressure given above, the ethoxy reaction does not take place. Ador and Victor Meyer decomposed diazobenzenesulphonic acid with ethyl alcohol about twenty years ago.[1] They found only the hydrogen product.

[1] Ann. Chem. (Liebig) **159**, 8.

Action of Propyl Alcohol on Paradiazobenzenesulphonic Acid at Ordinary Pressure.

5 grams of the diazo compound and 50 cc. propyl alcohol, boiling-point 96°, were placed in a small flask connected with an inverted condenser. The flask was placed in a bath of a concentrated solution of sodium chloride. The bath was heated to boiling for two hours. The decomposition may have been complete, and probably was, before this time, but owing to the fact that the diazo compound contained some sulphanilic acid which had escaped the diazotizing action, and that the product of the decomposition was a black tarry liquid, it was difficult to tell accurately when the decomposition was complete. The excess of propyl alcohol was distilled off, the tarry residue diluted with water and filtered. The solution filtered with great difficulty, and a residue was left on the filter which was not sulphanilic acid. The filtrate was a muddy-looking liquid, holding something in suspension which could be removed only by repeated filtering. Upon adding a solution of barium hydroxide to neutralize the acid or acids formed, and to precipitate the sulphuric acid formed from the decomposition of the sulphonic acid, a tarry deposit was obtained. This was filtered off, and carbon dioxide passed into the solution to precipitate the excess of barium hydroxide. The barium carbonate was then filtered off and the solution boiled to decompose any acid barium carbonate which might have been formed. Sodium carbonate was then added, and after filtering, the solution was evaporated to dryness. The sodium salt was of a dark brown color. This was treated with phosphorus pentachloride. The residue obtained after driving off the phosphorus oxychloride was a black oily liquid weighing 2 grams. This was treated with an excess of concentrated ammonium hydroxide. After evaporating off the excess, a black gummy substance was obtained. This was dissolved in hot water and treated with animal charcoal, small quantities only being used. It did not crystallize well from water; mixed with small white crystals there was always found a dark gummy substance. This mixture was recrystallized from alcohol, the alcohol removed, and the crystals redissolved in hot water. By this means they can be obtained pure. An examination showed them to be the crystals of benzenesulphonamide. No other amide was found.

When paradiazobenzenesulphonic acid is decomposed with propyl alcohol, a much more serious breaking down occurs than when the decomposition is effected with either methyl or ethyl alcohol. The amide, or rather the acid from which the amide is derived, can be obtained only in very small quantities, while the principal product of the decomposition, so far as quantity is concerned, is the tarry matter referred to above.

Action of Propyl Alcohol on Paradiazobenzenesulphonic Acid at a Pressure of 700 mm.

The object of this experiment was to determine what effect a higher pressure than that used in the preceding experiment would have on the decomposition products. I desired to effect the decomposition at a pressure of about 840 mm. The experiment was carried out in the usual way. Upon applying heat the decomposition began almost immediately, and before the pressure could be raised to 840 mm. it was complete, the manometer indicating 700 mm. The product of this action was a black tarry liquid. It was purified with animal charcoal, then treated with barium hydroxide and converted into the amide as above. But *one* amide, benzenesulphonamide, was formed, and that in very small quantity.

The results obtained with ethyl and propyl alcohols are in accordance with those on record. Experiments with other alcohols were not made, the results obtained being considered sufficient for the purposes of this investigation.

The acid of which methoxybenzenesulphonamide is a derivative has been prepared by Kekulé,[1] and by Cahours and Annaheim ;[2] and some of its derivatives by Haitinger.[3] I desired to prepare the acid in the manner described by Kekulé from anisol (methylphenyl ether) and sulphuric acid, in order that the amide made from it might be compared with the amide obtained from the acid formed by the decomposition of diazobenzenesulphonic acid with the methyl alcohol.

Preparation of Anisol.—In view of the observations of Remsen and Orndorff[4] that phenetol is the principal product of the action of ethyl alcohol on diazobenzene nitrate, it was thought probable that anisol would be formed by the action of methyl alcohol.

[1] Ztschr. f. Chem. **1867**, 201.
[2] Monatshefte **4**, 173.
[3] Ann. Chem. (Liebig) **172**, 47.
[4] This Journal **9**, 387.

This was found to be the case, and indeed the method can be recommended for the preparation of anisol.

The diazo compound and absolute methyl alcohol were heated together in a flask connected with an inverted condenser. The decomposition began almost immediately, and after the temperature had been raised to 45° the burner was removed. The decomposition continued without further application of heat. I afterwards found that the decomposition will take place as well without the application of heat. If methyl alcohol and diazobenzene nitrate be brought together and allowed to stand, decomposition begins in 10–15 minutes, with elevation of temperature. After the action was complete the contents of the flask were subjected to distillation. Anisol and methyl alcohol passed over. The alcohol had a yellow color and an aromatic odor, due to the presence of anisol. This distillate was treated with about six times its volume of a saturated solution of sodium chloride. On standing, a light yellow oil collected at the surface. In order to obtain the last portions of anisol, the sodium-chloride solution was distilled; methyl alcohol and a small quantity of anisol passed over, but in this case the oil collected in the bottom of the receiver in globules and could easily be obtained. To the contents of the flask from which the anisol and methyl alcohol had been obtained in the first instance, sodium hydroxide was added to alkaline reaction. The flask was then heated and steam passed into it, when a small quantity of anisol passed over with the water vapor. Sulphuric acid was then added to the mixture in the flask to acid reaction and it was again subjected to distillation with steam. Orthonitrophenol was formed in the receiver and condenser. The residue in the flask was not examined further. The anisol obtained was purified by treating with fused calcium chloride and then distilled. It boiled at 151°.

Action of Sulphuric Acid on Anisol.—The anisol was poured slowly into an excess of ordinary concentrated sulphuric acid. It apparently dissolved, with a slight elevation of the temperature of the mass. It was then heated for half an hour on the water-bath. The mixture was neutralized with barium carbonate and converted into the potassium salt. This solution was evaporated to dryness. The dried substance is exceedingly hard, brittle and hygroscopic. To preserve it in the dry condition it must be kept in a desiccator. This potassium salt was converted into the sulphonic chloride by treatment with phosphorus pentachloride. At the ordinary tem-

perature no action takes place, and it is necessary to apply heat. After distilling off the phosphorus oxychloride the residue was treated with water. No oil was observed. I then added ether in order to extract any sulphonic chloride which might have been formed. Upon pouring off the ethereal solution and distilling, beautiful crystal plates, of a pleasant odor, somewhat suggestive of the sulphonic chlorides in general, were obtained. These were supposed to be the crystals of either ortho- or paraanisolsulphonic chloride. The orthochloride melts at 55° (Haitinger). The crystals that I obtained melted at 86°. The following analysis of the compound was then made:

I. .10825 gram of the substance gave .1023 gram of silver chloride, corresponding to 23.37 per cent. of chlorine.

II. .23469 gram of the substance gave .21665 gram of silver chloride, corresponding to 22.83 per cent. of chlorine (Carius' method).

I. .2184 gram of the substance gave .2244 gram of carbon dioxide, corresponding to 28.02 per cent. of carbon.

II. .2880 gram of the substance gave .0694 gram of water, corresponding to 2.67 per cent. of hydrogen.

I. .23469 gram of the substance gave .3623 gram of barium sulphate, corresponding to 21.21 per cent. of sulphur (Carius' method).

For $C_6H_4.OCH_3.SO_2Cl$:

		Theory.	Found. I.	Found. II.
C_7	83.79	40.60	28.02	...
H_7	7.00	3.39	...	2.67
O_3	47.88	...	...	...
S	31.98	15.52	21.21	...
Cl	35.37	17.17	23.37	22.83
	206.02			

Evidently the compound analyzed was not anisolmonosulphonic chloride. It was the disulphonic chloride. This result was unexpected, since Zervas,[1] in order to make the disulphonic acid, treated anisol with *fuming* sulphuric acid. I obtained it as described above by using ordinary concentrated acid and heating for a half-hour on the water-bath.

Analysis for $C_6H_3.OCH_3(SO_2Cl)_2$:

[1] Ann. Chem. (Liebig) **103**, 342.

		Theory.	Found. I.	II.
C_7	83.79	27.54	28.02	...
H_6	6.00	1.97	...	2.67
O_8	79.80	...	...	...
S_2	63.96	21.02	21.21	...
Cl_2	70.74	23.25	23.37	22.83
	304.29			

Lack of time prevented me from repeating the experiment with anisol and sulphuric acid. A portion of the disulphonic chloride obtained as described above was converted into the amide by treatment with ammonium hydroxide. Upon crystallizing the product, apparently crystals of two different kinds were found. They were not investigated.

Wichelhaus[1] states that if benzenesulphonamide be warmed with phosphorus pentachloride, benzenesulphondichlorphosphamide is formed. It was desirable to know how methoxybenzenesulphonamide would act under similar conditions. The following experiment was made to determine this action:

Two grams of methoxybenzenesulphonamide were treated with the proper proportion of phosphorus pentachloride. No action took place at the ordinary temperature. Upon applying heat, action began at once, and hydrochloric acid was given off. The oily product obtained was poured on a porous plate and allowed to stand over sulphuric acid for about two weeks (Wichelhaus). It was then dissolved in anhydrous ether and allowed to stand. Although several attempts were made to obtain crystals, they were unsuccessful. When the ether was allowed to evaporate off slowly, a clear viscous liquid was obtained, which, upon being exposed to the air, gave off hydrochloric acid. When the product of the decomposition of the amide and phosphorus pentachloride was allowed to stand in moist air, or when water was added to it, decomposition began at once and the substance was converted back to the amide. In this respect its behavior is similar to that of benzenedichlorphosphamide.

Action of Nitric Acid on Methoxybenzenesulphonamide. — 4.7 grams of methoxybenzenesulphonamide were added slowly to 15 cc. of fuming nitric acid. The action was very energetic. For this reason only a small quantity of the amide should be added at a time. Upon diluting the nitric acid solution with water

[1] Ber. d. chem. Gesell. **2**, 502.

a precipitate or emulsion of minute globules of oil was obtained. The solution was diluted still further and heated to boiling. The oil dissolved with difficulty. Upon cooling, beautiful crystals of slender needle-shape, somewhat similar to those of the amide, separated. A determination of the melting-point, which was found to be 88°, showed that the substance was not the amide. A test for sulphur failed to show its presence. This, together with the form of the crystals, led me to believe that the substance was metadinitrobenzene, and this proved to be the case.

The melting-point was 88° (uncorrected). That of metadinitrobenzene is 89.9° (corrected).[1] Its solubility in hot water, alcohol and ether agreed with that of metadinitrobenzene in these reagents.

The preceding experiment was repeated, a more dilute acid—ordinary concentrated nitric acid—being employed. The action was not quite so energetic, but otherwise the same results were obtained. With more dilute acid the reaction does not take place. The amide dissolves, but, on cooling, it crystallizes out unchanged. These experiments show that the paramethoxybenzenesulphonamide is very susceptible to the action of nitric acid. Not only are the methoxy and sulphonamide groups, which are in the para position, replaced by the nitro group, but apparently a rearrangement of the parts takes place, the nitro groups entering the *meta* position.

Summary of Results.

I. When paradiazobenzenesulphonic acid is decomposed with methyl alcohol under diminished pressure, only the hydrogen product is obtained.

II. At ordinary pressure both the hydrogen and methoxy reactions take place.

III. At a pressure of 350 mm. both reactions take place.

IV. At a pressure of 850 mm. both reactions take place, the methoxy compound predominating.

V. At a pressure of about 30 atmospheres the methoxy reaction alone takes place.

VI. When ethyl alcohol is used in the decomposition of paradiazobenzenesulphonic acid, at various pressures up to 850 mm., only the hydrogen product is obtained.

VII. When propyl alcohol is used under the same conditions the hydrogen product only is formed.

[1] Griess: Berichte **11**, 625.

THE PROTEIDS OF THE WHEAT KERNEL.

By Thomas B. Osborne and Clark G. Voorhees.

Wheat flour has for centuries formed one of the most important articles of food for man, in consequence of the peculiar property which it possesses, in common only with rye flour, of forming a dough when mixed with water which on leavening and baking produces a light, porous bread. This characteristic depends wholly on the proteid constituents of the seed.

The characters, composition, and even the number of the proteids contained in the wheat kernel or derived from it, are the subject of very conflicting statements on the part of those who have investigated these bodies. It was with a hope of clearing away much of the existing confusion, by applying the modern methods of study, that this investigation was undertaken. The amount of work involved has been very great, and consequently its record is long; but it was deemed best to publish the results in full detail, as otherwise a clear comprehension of what we have done and the value of our conclusions would be impaired.

I.—Historical.

Beccari[1] appears to have been the first to publish the fact that by washing with water a dough made of wheat flour, as long as starch is removed, a coherent, elastic mass is obtained.

Einhof[2] found that hot alcohol extracts from wheat flour a substance which is precipitated by cooling, by dilution, or by concentration. This he considered to be the same substance as the gluten (*Kleber*) obtained by washing the flour with water.

Taddei[3] found that on boiling gluten with alcohol a part is dissolved. The residue left by thus treating the gluten he named *zymom;* the part soluble in alcohol, *gliadin.*

Berzelius[4] believed to have found in the alcoholic extract of the gluten another proteid substance which he called mucin. This was obtained by treating the proteid extracted by alcohol from gluten with acetic acid, the mucin remaining as a jelly undissolved. Berzelius[5] considered that Taddei's zymom and gliadin were

[1] Common. Bonon. **1**, 1, 122.
[2] Jour. d. Chemie von Gehlen **5**, 131, 1805.
[3] Annals of Phil., May, 1820, and Abstract, Schweiger's Jour. f. Chem. u. Physik. **29**, 514.
[4] Lehrbuch d. Chemie, 3. Aufl. **6**, 453.
[5] Berzelius Jahresb. **7**, 231, 1826.

substances already known, that the gliadin was identical with the body obtained by Einhof from wheat, rye, and barley. His own investigation also indicated that Taddei had applied new names to known and common constituents of seeds. By diluting the alcoholic extract of gluten with water and concentrating, a substance separated on cooling the residual aqueous solution, having the same nature as that separated by Einhof in a similar way from rye and barley. The substance undissolved by alcohol Berzelius found to be so similar to animal albumin that the two could not be distinguished in appearance, and therefore called it *plant albumin.*

De Saussure[1] found wheat gluten to contain about 72 per cent. of plant albumin in the insoluble form, about 20 per cent. of plant gelatin, or as he proposed to call it, *glutin*, and about 1 per cent. of mucin.

This latter substance he regarded as similar to the mucin described by Berzelius, though prepared in a different manner. It was obtained by extracting the gluten with hot alcohol, separating the plant gelatin or glutin by adding an equal volume of water, and concentrating to one-sixth. On standing, and by repeatedly adding water and evaporating until the liquid remained clear on cooling, a solution was obtained which, evaporated to dryness, yielded mucin in an impure form.

This body De Saussure supposed had the power to convert starch into sugar.

Boussingault,[2] like Einhof, considered the portion of gluten soluble in alcohol to be the same as the entire gluten proteid. He gave analyses of four preparations: **1.** The washed gluten; **2.** The proteid extracted by alcohol; **3.** Preparation **2** dissolved in acetic acid and precipitated by carbonate of ammonia; **4.** Albumin coagulated by heating the water-washings of the gluten after the starch had been separated.

	1	**2**	**3**	**4**
Carbon	53.5	54.2	52.3	52.7
Hydrogen	7.0	7.5	6.5	6.9
Nitrogen	15.0	13.9	18.9	18.4
Oxygen	24.5	24.4	22.3	22.0
	100.0	100.0	100.0	100.0

[1] Schweiger's Jour. **69**, 188, 1833.
[2] Ann. de chim. et de phys. **65**, 30. Abstract, Berzelius Jahresb. **18**, 327, 1837.

Berzelius, commenting on the above analyses, remarked that the lower content of nitrogen in **2** as compared with **3** shows that the mucin which is contained in it has a lower nitrogen content than plant gelatin, and that Boussingault evidently did not know of the existence of mucin.

Liebig,[1] in a paper on the nitrogenous food substances of the vegetable kingdom, discussed the relation of vegetable proteids to those of animal origin. In wheat gluten he found a substance insoluble in alcohol, Taddei's zymom and Berzelius' plant albumin, which he named plant fibrin, on account of its agreement both in composition and properties with blood fibrin. He rejected Berzelius' name of plant albumin, as solubility in water is an essential characteristic of albumins. The substance extracted from the gluten by alcohol he called plant gelatin, and considered it to be a casein-like compound of a proteid with an organic acid, whose nature, however, he was unable to discover. He mentioned De Saussure's mucin, saying that its composition must be nearly the same as that of plant fibrin, since the composition of the crude gluten varied little from that of plant fibrin and albumin. The aqueous extract of the flour also contained albumin. He then gave the following analyses of these various substances, made in his laboratory by Jones, Will and Verentrap, and Scherer.

Plant Albumin.

	From Wheat.	From Plant Gelatin. Will & Verentrap.
Carbon	55.01	54.85
Hydrogen	7.23	6.98
Nitrogen	15.92	15.88
Oxygen, Sulphur, Phosphorus	21.84	22.39
	100.00	100.00

Plant Gelatin.

	Jones.		
	Crude.	Extracted with Ether.	Ditto. purified.
Carbon	58.45	56.80	55.22
Hydrogen	7.65	7.60	7.42
Nitrogen	..	..	15.98
Oxygen	..	..	21.38
			100.00

[1] Ann. der Chem. u. Pharm. **39**, 129, 1841.

Plant Fibrin.

	Jones.	Scherer.	
Carbon	53.83	54.603	54.603
Hydrogen	7.02	7.302	7.491
Nitrogen	15.59	15.810	15.810
Oxygen, Sulphur, Phosphorus	23.56	22.285	22.096
	100.00	100.00	100.00

In these analyses, nitrogen was determined by finding the ratio of nitrogen to carbonic acid in the products of combustion, and then calculating the percentage of nitrogen from that of the carbon. Scherer[1] prepared plant fibrin by dissolving gluten in dilute potash water, filtering, neutralizing with acetic acid, extracting the precipitate with boiling alcohol as long as anything was removed, exhausting with ether and drying at 100°. The analysis of the preparation thus obtained, after recalculating the figures given by Scherer for the present atomic weight of carbon, was as follows:

Carbon	53.24
Hydrogen	7.13
Nitrogen	15.36
Sulphur, Oxygen, Phosphorus	25.17
	100.00

Bouchardat[2] considered wheat gluten to contain a body, named by him albuminose, which he described as a substance soluble in extremely dilute acids, forming the chief constituent of egg albumin, blood fibrin, casein, and grain gluten.

Dumas and Cahours[3] found four proteid substances in flour. After extracting gluten with alcohol, a gray, fibrous residue was obtained, which one of the authors had previously designated in 1839 as plant fibrin. The alcoholic solution on cooling deposited a proteid having the characteristics of casein. From the remaining solution, after concentration and cooling, a substance separated which they, like De Saussure, called *glutin*.

The aqueous solution obtained in washing out the gluten contained a body similar to albumin.

The fibrin, they say, had up to that time been considered as coagulated albumin, and the same composition had been assigned

[1] Ann. d. Chem. u. Pharm. **40**, 7, 1841. [2] *Ibid.* **43**, 124, 1842.
[3] Jour. f. prakt. Chem. **28**, 398, 1843.

to both. They found by careful analysis that the two bodies had a different composition. The fibrin freed from all substances soluble in alcohol and ether, soaked in water, and treated with diastase, to remove starch, had the following composition:

	1.	2.
Carbon	53.23	53.37
Hydrogen	7.01	7.02
Nitrogen	16.41	16.00
Oxygen, etc.	23.35	23.61
	100.00	100.00
Ash	1.06	

In this analysis nitrogen was determined by Dumas' method, and carbon and hydrogen calculated for an atomic weight of 12.

As this analysis gave nearly the same composition as blood fibrin, the authors considered the two bodies to be identical.

They obtained coagulated albumin by boiling and concentrating the aqueous washings of the gluten. After treating the coagulum with diastase and extracting with alcohol and ether, it was dried at 140° and analyzed. Its composition was thus found to be:

Carbon	53.74
Hydrogen	7.11
Nitrogen	15.65
Oxygen, etc.	23.50
	100.00
Ash	8.50

This analysis, they said, shows that the albumin is identical with egg albumin. They found, however, that on prolonged boiling the fibrin yielded a substance of the same composition as coagulated albumin, and they therefore assumed that by heating with water, albumin was split off from the fibrin and coagulated.

They then suggested that the albumin may have resulted from a decomposition of the fibrin which had dissolved on washing out the gluten.

The casein obtained by cooling the weak alcohol extract of the gluten, after washing with water, alcohol and ether, and drying *in vacuo*, gave the following results on analysis:

Carbon	53.46
Hydrogen	7.13
Nitrogen	16.04
Oxyen, etc.	23.37
	100.00

The proteid, called by the authors glutin, remaining in the alcoholic solution after deposition of the casein, was separated from solution by concentration to small volume, and, after drying, extracted with ether. When dried *in vacuo* at 140° they found it contained:

Carbon	53.27
Hydrogen	7.17
Nitrogen	15.94
Oxygen, etc.	23.62
	100.00

This, they claimed, showed the glutin to be isomeric with albumin and casein.

Mulder[1] extracted plant gelatin from gluten by means of alcohol. The solution was filtered hot, allowed to cool, and the white, flocculent precipitate which deposited was again dissolved in alcohol and twice separated by cooling, filtered off, extracted with ether, dried, and analyzed with the following result:

	I.	II.
Carbon	54.94	54.75
Hydrogen	7.11	6.99
Nitrogen	15.71	15.71
Sulphur	0.57	0.62
Oxygen	21.68	21.93
	100.00	100.00

This plant gelatin he considered to be a compound of sulphur with "protein," containing the same proportion of sulphur as blood albumin.

Von Bibra[2] states that by boiling gluten with diluted alcohol, insoluble plant fibrin remains undissolved, while plant gelatin and plant casein go into solution, the latter separating on cooling. These three bodies he found, after examining seven different samples of gluten, to be present in the following average proportions:

Plant fibrin	70.80
Plant gelatin	16.22
Plant casein	7.10
Fat	5.88
	100.00

[1] Ann. d. Chem. u. Pharm. 52, 419, 1844. [2] Die Getreidearten u. das Brod, 1860.

He assigns the following composition to each of these proteids:

	Plant Fibrin.	Plant Gelatin.	Plant Casein.
Carbon	53.57	53.57	54.88
Hydrogen	6.95	7.12	7.05
Nitrogen	15.70	15.57	15.71
Sulphur	1.02	0.88	0.76
Oxygen	22.76	22.86	21.60
	100.00	100.00	100.00

Von Bibra considers these bodies to have the same elementary composition and to be "isomers in the narrower sense of the term."

In the water used for washing out the gluten he found a coagulable proteid which he calls albumin. Dried at 110°, this coagulated proteid formed 1.34 per cent. of the flour and contained 15.65 per cent. of nitrogen.

Günsberg[1] found that on boiling gluten or plant gelatin with water a considerable part dissolved, which on cooling mostly separated. He says that the body previously called plant casein is a mixture of plant gelatin with the insoluble part of the gluten which has escaped separation on filtering, and that Taddei's view is correct that there are but two bodies contained in gluten. By boiling gluten with water he obtained five preparations, the average of whose composition was as follows:

Carbon	52.68
Hydrogen	6.77
Nitrogen	17.76
Oxygen	22.79
	100.00

In the same way from gliadin he obtained a body of the same composition, namely,

Carbon	52.65
Hydrogen	6.88
Nitrogen	17.45
Oxygen	23.02
	100.00

Commaille[2] recognized as proteid constituents of flour: 1. sitosin, soluble in water and coagulable by heat; 2. imesin, dissolving

[1] Jour. f. prakt. Chem. **85**, 213, 1862. [2] J. Pharm. (4) **4**, 108, 1866.

after drying only in water containing 0.1 per cent. hydrochloric acid; 3. sitesin, dissolving in water containing 0.1 per cent. hydrochloric acid; 4. glutin, nearly insoluble in acidulated water, sparingly soluble in acetic acid, easily in strong acid, not miscible with water, forming an emulsion with alcohol which is separated by a large quantity of water and also by ether. Thus precipitated it dries to a translucent, white, friable mass slightly soluble in water. 5. Mucin, dissolves easily, even after drying, in water and cold alcohol of 80 per cent.

Ritthausen[1] recognizes five proteids in wheat, namely, gluten casein, gluten fibrin, plant gelatin or gliadin, mucedin, and albumin. The existence of the latter he considers somewhat doubtful, as it may possibly be a mixture of mucedin and gliadin. He formerly[2] considered Dumas' casein, or as he proposed to call it paracasein, to be a constituent of the gluten. The four bodies first named form the gluten, the first being insoluble in cold dilute alcohol, the others soluble in that menstruum. The albumin he obtains from the water used in washing out the gluten, by acidifying slightly with nitric acid and boiling. The gluten casein he prepared by thoroughly extracting the gluten with dilute alcohol, dissolving the residue in dilute potash water, precipitating with acetic acid, extracting the washed precipitate with dilute alcohol, redissolving the precipitate in dilute potash water, filtering, and again precipitating with acid.

The bodies soluble in alcohol are separated from each other by fractional precipitation from alcoholic or acetic-acid solution, the precipitations being repeated until the products have a constant composition as follows:

	Gluten Casein.	Gluten-Fibrin.	Gliadin.	Mucedin.	Albumin.
Carbon	52.94	54.31	52.76	54.11	53.12
Hydrogen	7.04	7.18	7.10	6.90	7.18
Nitrogen	17.14	16.89	18.01	16.63	17.60
Sulphur	0.96	1.01	0.85	0.88	1.55
Oxygen	21.92	20.61	21.37	21.48	20.55
	100.00	100.00	100.00	100,00	100.00

Weyl and Bischoff[3] consider that the proteid matter of wheat is chiefly a myosin-like globulin which they call vegetable myosin,

[1] Die Eiweisskörper, etc. Bonn, 1872. [2] J. prakt. Chem. **85**, 193.
[3] Ber. d. deutsch. chem. Gesell. 1880, 367.

and that if so, this must be the substance from which gluten is derived, for other proteids are present only in small quantity. Extraction with 15-per cent. salt solution left a residue from which they obtained no gluten. They therefore considered it probable that the gluten forms from the myosin in consequence of a ferment action similarly to the formation of fibrin from fibrinogen. No ferment, however, could be detected. They also found that large amounts of sodium chloride, sodium sulphate and magnesium sulphate hindered the formation of gluten in the same way that sodium and magnesium sulphates hinder the formation of fibrin. As no gluten was obtained from flour extracted with alcohol, they concluded that the myosin had been coagulated. By warming flour 48–96 hours below 6°, the coagulation-point of myosin, no gluten was obtained from the meal after adding a little unwarmed flour, showing that the gluten-forming substance had been coagulated.

According to Martin,[1] alcohol extracts from gluten but one proteid substance. This is soluble in hot water, but insoluble in cold, hence is insoluble phytalbumose. The residue remaining after treatment with alcohol is uncoagulated proteid, soluble in dilute acids and alkalies; this he called gluten fibrin. The insoluble phytalbumose is not present as such in flour, since direct extraction of the meal with 75-per cent. alcohol removes no proteid. Extraction with water yields less globulin and soluble albumose than extraction with sodium-chloride solution of 10–15 per cent. Martin therefore concluded that the insoluble phytalbumose is formed from the soluble by the action of water, the gluten fibrin being formed by a similar action of water on the globulin, that is, conversion into an albuminate. This albuminate and the insoluble phytalbumose together constitute gluten.

W. Johannsen[2] believes that there is no ferment action in the formation of gluten. Dough was obtained by grinding dried gluten and mixing with starch, and also by mixing moist gluten with starch.

Chittenden and Smith[3] prepared many samples of gluten casein according to Ritthausen's method. As an average of eight analyses they give the following composition:

[1] British Medical Jour. **2**, 104, 1886.
[2] Ann. Agromom. **14**, 420, 1888. Abstract, Jour. Chem. Soc., March, 1889.
[3] Jour. Physiol. **11**, 419, 1890.

Carbon	52.87
Hydrogen	6.99
Nitrogen	15.86
Sulphur	1.17
Oxygen	23.11
	100.00

The wheat used in the work now to be described was of two kinds. One of these, Scotch Fife, a spring wheat, was obtained through the kindness of Dr. D. N. Harper, late Chemist of the Minnesota Experiment Station. It was carefully selected, free from all other varieties, and was milled under the supervision of Dr. Harper, who supplied us with samples of the various mill products, together with some of the unground wheat. Two grades of flour were used, namely, "patent flour," made from the finest and purest middlings, and "straight flour," from the coarser and less pure middlings. The "shorts," chiefly composed of the outer coating of the seeds together with adhering portions of the endosperm, was also examined. Samples of whole wheat flour were prepared as required from the wheat by grinding small quantities in the laboratory mill. The other wheat used was a variety of winter wheat known as "Fultz." This was procured from a seedsman, was carefully selected and freshly harvested. Portions of this wheat were ground in the laboratory mill as needed, and only whole wheat flour was obtained from this sample. Preliminary experiments showed that all these different flours yielded proteid matter to diluted alcohol, to water, to 10-per cent. sodium-chloride solution, and after complete and successive extraction with these several reagents, to dilute potash water. The bodies extracted by these reagents will now be considered separately.

II.—Proteids Soluble in Water.

a.—Preliminary Experiments.

200 grams of the "straight flour" from spring wheat were poured with constant stirring into 800 cc. of distilled water. No coherent gluten formed, the undissolved flour settling down as a loose mass. After a few hours' digestion the solution was filtered and the residue collected on a filter. When most of the solution had run off the residue began to form a tough, glutinous mass, which increased in density as the excess of solution separated.

The water-extract was of a straw-yellow color, becoming red-brown on standing, and had a *very* slight acid reaction.

Saturated with ammonium sulphate, a bulky precipitate formed, which on standing contracted, showing the solution to contain but a small amount of proteid matter. After 24 hours this precipitate was completely soluble in water, giving no evidence of the formation of so-called albuminates. Saturation with sodium chloride gave a small precipitate. Acetic acid in the cold gave no precipitate until sodium chloride was added.

On slowly heating the solution gave a turbidity at 48° and a flocculent coagulum at 52°. After heating to 65° for some time and filtering, the solution became turbid again at 73°, flocks forming in very small amount at 82°. No more separation occurred on further heating the extract even to boiling. The addition of a little acetic acid and sodium chloride gave a small precipitate. The body coagulating at 52° formed the greater part of the proteid in solution. The complete coagulation of this proteid was accomplished with difficulty, prolonged heating at 65° being necessary to cause it to separate completely. The addition of sodium chloride greatly facilitated the final coagulation. The temperature at which the flocculent coagulum separated depended upon the rate of heating. Unless the solution was heated very slowly, the point at which flocculation occurred was found to be much above 52°.

As the subsequent experiments showed that these same proteids went into solution on extracting the flour with 10-per cent. sodium-chloride brine, the further examination of the water-soluble substances was confined to extracts originally made with 10-per cent. sodium-chloride solution after separation of the globulins by dialysis.

Again, 4000 grams of straight flour were treated with 8 liters of 10-per cent. sodium-chloride solution, strained through a sieve to break up all lumps, and allowed to settle over night. Only about one-half the extract could be separated from the undissolved flour by decantation and filtration. The residue was stirred up with 2 liters more of the same solution, and again thrown on to filters. The filtrate was collected in successive portions and saturated with ammonium sulphate as fast as obtained. The precipitate so produced was filtered off, treated with 10-per cent. brine, the resulting solution filtered clear, and dialyzed until chlorides

were removed. The globulin which was thus precipitated was filtered off, and the solution again dialyzed for 14 days, but no more globulin could be obtained.

On slowly heating a portion of this solution in a double water-bath, turbidity occurred at 48°, flocks separating at 55°. After heating for some time at 65° the coagulum was filtered off and the solution again heated, a turbidity resulting at 70°, and a very minute amount of flocculent coagulum at 80°. Boiling the solution after filtering gave no more precipitate, and nothing was obtained by adding a *little* salt and acetic acid. If the amount of salt was increased and acetic acid added, a precipitate was obtained. Equal volumes of this solution were treated with 20 per cent. of sodium chloride and a little acetic acid. To the first the salt and acid were added directly, to the second after heating to 65° and filtering off the coagulum, and to the third after heating to 95° and likewise filtering. The first portion gave the most precipitate, the last the least, showing that the coagulable proteids are thus precipitated. The filtrate from the first portion when neutralized and boiled gave no precipitate, showing that the separation of the albumin was complete. This result was to be expected, for albumin may be thus precipitated by salt and acid.

This solution freed from globulins by dialysis gave a precipitate on saturation with sodium chloride, the filtrate from which became turbid when heated to 43°, flocculent at 56°, and no more precipitation on further heating, showing that the higher-coagulating proteid was thus removed. This dialyzed solution likewise gave a considerable precipitate with nitric acid. On heating a part remained insoluble, and on filtering this off the filtrate gave a precipitate on cooling, which dissolved again on heating and reappeared as often as the solution was cooled. The filtrate from the salt and acid precipitate did not give this reaction, but the solution of the precipitate in water gave it very strongly. This reaction is characteristic of some proteoses, and shows that the salt and acid precipitate contains a proteose together with the albumins. This proteose is likewise precipitated by saturating the extract with salt, for on dissolving the precipitate so produced, and separating the albumin contained in it by coagulation, the filtrate gave a strong *red* biuret reaction, and a heavy precipitate with nitric acid which dissolved on warming and precipitated again on cooling. The filtrate from the precipitate caused by saturation with salt gives

no reaction with nitric acid, showing that the proteose is thus completely precipitated.

Saturation of the solution with sodium chloride seems to render the higher-coagulating proteid insoluble, for an aqueous solution of the precipitate shows only the presence of the low-coagulating albumin, and the filtrate from the salt saturation precipitate likewise coagulates only at the lower temperature.

We are able then to recognize three distinct proteid substances soluble in pure water, namely, two coagulable, presumably both albumins, and a proteose. As it was found that the proteid removed from the flour by treatment with alcohol was to a slight extent soluble in pure water, it might be thought that one of these bodies was identical with that. The identity with the two albumins is excluded by the fact that they are precipitated by heat, and with the proteose by the fact that the alcohol-soluble proteid gives a precipitate with hydrochloric acid when dissolved in distilled water and also with a trace of sodium chloride, which the proteose does not.

In order to be sure that the body, which was apparently an albumin, was not a myosin-like globulin held in solution by the small amount of salts contained in the river water used for dialysis, as was suggested by its partial precipitation by saturation with sodium chloride, the following experiment was tried: 250 cc. of a strong aqueous extract of winter wheat meal was made and placed in a dialyzer immersed in distilled water. Distilled water was then allowed to run through the outer vessel continuously for 48 hours. A small precipitate was then formed, which was filtered off, the clear solution returned to the dialyzer, and the process continued for five days longer. No more substance separated. The entire solution, which was still found to coagulate at 54°, was then evaporated to dryness in a weighed platinum dish, the considerable proteid residue burned off, and the total mineral matter weighed. It was found to amount to only 0.0008 gram, thus proving that the substance was an albumin.

b.—Albumins.

The remainder of the foregoing dialyzed solution which was left after the preliminary trials preceding the last, formed about five-sixths of the whole. This was then heated in a large water-bath to a temperature not exceeding 61°, the water in the bath being

kept between 60° and 65°. After an hour the precipitate was filtered off, washed thoroughly with water, alcohol, absolute alcohol and ether, and dried. Before drying the coagulum was a white, voluminous, semi-solid mass, which when completely dried over sulphuric acid became dense and horny.

4.3 grams of this preparation were obtained, and after drying at 110° it was found to have the following composition:

COAGULATED WHEAT ALBUMIN. *Preparation* 1.

	I.	II.	Average.	Ash-free.
Carbon	53.04	53.28	53.16	53.27
Hydrogen	6.74	6.89	6.82	6.83
Nitrogen	16.86	16.95	16.91	16.95
Sulphur	1.27	..	1.27	1.27
Oxygen	..	..	..	21.68
Ash	0.22	..	..	..
				100.00

Ash.—0.6479 gram dried substance gave 0.0014 gram ash = 0.22 per cent.

Carbon and Hydrogen, I.—0.3570 gram dried substance gave 0.6943 gram CO_2 = 53.04 per cent. C, and 0.2165 gram H_2O = 6.74 per cent. H.

II.—0.4062 gram dried substance gave 0.7937 gram CO_2 = 53.28 per cent. C, and 0.2522 gram H_2O = 6.89 per cent. H.

Nitrogen, I.—0.3789 gram dried substance gave 51.14 cc. N at 5° (barometer 773 mm. at 23°) = 16.86 per cent. N.

II.—0.3764 gram dried substance gave 53.15 cc. N at 6° (barometer 745.3 mm. at 22°) = 16.95 per cent. N.

Sulphur.—0.8292 gram dried substance gave 0.0769 gram $BaSO_4$ = 1.27 per cent. S.

Another lot of 10,000 grams of flour was extracted with 10-per cent. sodium-chloride solution and treated in the same way as the extract last described. The albumin was obtained from the extract after completely removing the globulins, by coagulation at 60°. This coagulum was treated in the same manner as preparation 1. It weighed 6.4 grams and had the following composition:

COAGULATED WHEAT ALBUMIN. *Preparation* 2.

	I.	II.	Average.	Ash-free.
Carbon	52.91	52.98	52.95	53.06
Hydrogen	6.86	6.74	6.80	6.82
Nitrogen	16.93	17.01	16.97	17.01
Sulphur	1.28	1.31	1.30	1.30
Oxygen	..	..	..	21.81
Ash	0.22	..	..	..
				100.00

Ash.—0.6376 gram substance dried at 110° gave 0.0014 gram ash = 0.22 per cent.

Carbon and Hydrogen, I.—0.4020 gram dried substance gave 0.7800 gram CO_2 = 52.91 per cent. C, and 0.2482 gram H_2O = 6.86 per cent. H.

II.—0.3880 gram dried substance gave 0.7536 gram CO_2 = 52.98 per cent. C, and 0.2362 gram H_2O = 6.74 per cent. H.

Nitrogen, I.—0.3825 gram dried substance gave 54.16 cc. N at 11° (barometer 756 mm. at 22°) = 16.93 per cent. N.

II.—0.3015 gram dried substance gave 42.23 cc. N at 11° (barometer 768 mm. at 23°) = 17.01 per cent. N.

Sulphur, I.—0.6785 gram dried substance gave 0.0632 gram $BaSO_4$ = 1.28 per cent. S.

II.—0.3707 gram dried substance gave 0.0354 gram $BaSO_4$ = 1.31 per cent. S.

The filtrate from preparation **2** was then heated to 75° and a small amount of coagulum obtained which was highly colored. This was filtered off and washed with alcohol, thereby removing much of the color. After drying over sulphuric acid, 0.65 gram was obtained, which on analysis gave the following results:

COAGULATED WHEAT ALBUMIN. *Preparation* **3.**

		Ash-free.
Nitrogen	16.91	16.94
Ash	0.18	..

Ash.—0.2149 gram substance dried at 110° gave 0.0004 gram ash = 0.18 per cent.

Nitrogen.—0.3234 gram dried substance gave 45.05 cc. N at 11° (barometer 767.2 mm. at 22°) = 16.91 per cent. N.

Another preparation was made from the same flour by extracting 10,000 grams with 10-per cent. sodium-chloride solution, filtering the extract and dialyzing at once. In this case the precipitation of the proteids with ammonium sulphate was omitted. After complete dialysis the solution was filtered from the globulin which had separated, and the solution heated at once to 90°. This temperature was high enough to precipitate both albumins, but as the albumin coagulating at 75°–80° formed but a mere trace of coagulum, its separation was in this case not attempted.

Dried over sulphuric acid, this preparation, which in all respects resembled **1** and **2**, weighed 16.5 grams, and when analyzed gave the following results:

COAGULATED WHEAT ALBUMIN. *Preparation* 4.

	I.	II.	Average.	Ash-free.
Carbon	52.86	..	52.86	53.02
Hydrogen	6.85	..	6.85	6.87
Nitrogen	16.21	16.20	16.21	16.26
Sulphur	1.20	..	1.20	1.20
Oxygen	..	..	..	22.65
Ash	0.32	..	0.32	..
				100.00

Ash.—0.5514 gram substance dried at 110° gave 0.0018 gram ash = 0.32 per cent.

Carbon and Hydrogen.—0.2918 gram dried substance gave 0.5656 gram CO_2 = 52.86 per cent. C, and 0.1800 gram H_2O = 6.86 per cent. H.

Nitrogen, I.—0.4049 gram dried substance gave 55.36 cc. N at 16° (barometer 763 mm. at 27°) = 16.21 per cent. N.

II.—0.3393 gram dried substance gave 46.07 cc. N at 16° (barometer 767.5 mm. at 23°) = 16.20 per cent. N.

Sulphur.—0.7326 gram dried substance gave 0.0643 gram $BaSO_4$ = 1.20 per cent. S.

Another preparation of albumin was made by extracting with 10-per cent. sodium-chloride solution 2000 grams of so-called "shorts" from the spring wheat flour. This substance consisted chiefly of particles of the outer coats of the seed to which more or less of the adjacent endosperm adhered. After three hours the extract was strained through a coarse cloth and squeezed out from the residue in a screw press. After the starch had settled, the nearly clear extract, which had a deep red-brown color, was syphoned off and saturated with ammonium sulphate. The precipitate thus produced was filtered off and dissolved in 10-per cent. sodium-chloride brine. The resulting solution filtered from all the insoluble matter was then dialyzed free from chlorides, the precipitated globulins filtered off, and the albumin contained in the solution separated by heating to 65°. The coagulum was then filtered off, washed with water, alcohol and ether, and dried at 110° for analysis. It was found to have the following composition:

COAGULATED WHEAT ALBUMIN. *Preparation* 5.

		Ash-free.
Carbon	52.36	52.71
Hydrogen	6.80	6.85
Nitrogen	16.62	16.73
Sulphur	1.34	1.34
Oxygen	..	22.37
Ash	0.67	..
		100.00

Ash.—0.3730 gram substance dried at 110° gave 0.0025 gram ash = 0.67 per cent.

Carbon and Hydrogen.—0.3067 gram dried substance gave 0.5889 gram CO_2 = 52.36 per cent. C, and 0.1870 gram H_2O = 6.80 per cent. H.

Nitrogen.—0.4305 gram dried substance gave 61.29 cc. at 19° (barometer 758.3 mm. at 23°) = 16.62 per cent. N.

Sulphur.—0.2314 gram dried substance gave 0.0225 gram $BaSO_4$ = 1.34 per cent. S.

SUMMARY OF ANALYSES OF COAGULATED WHEAT ALBUMIN.

	1.	2.	3.	4.	5.	Average.
C	53.27	53.06	..	53.02	52.71	53.02
H	6.83	6.82	..	6.87	6.85	6.84
N	16.95	17.01	16.94	16.26	16.83	16.80
S	1.27	1.30	..	1.20	1.34	1.28
O	21.68	21.81	..	22.65	22.27	22.06
	100.00	100.00		100.00	100.00	100.00

The agreement of these figures is satisfactory with the exception of the nitrogen in 4. The accuracy of this analysis in this respect, however, cannot be doubted, as four determinations of this element were made, all of which agreed closely. As this preparation was separated at a higher temperature than any of the others, it is possible that it had in consequence lost some of its nitrogen. So far as the writer can find, none of the analyses heretofore made which professed to give the composition of wheat albumin agree with the above, as shown by the following table:

EARLIER ANALYSES OF WHEAT ALBUMIN.

	Boussingault.	Jones.	Dumas and Cahours.	Adriani.	Ritthausen.
C	52.7	55.01	53.74	54.78	53.12
H	6.9	7.23	7.11	7.34	7.18
N	18.4	15.92	15.65	16.01	17.60
S	22.0	21.84	23.50	21.87	1.55
O					20.55
	100.0	100.00	100.00	100.00	100.00

The imperfect methods of analysis used by Boussingault and Jones are sufficient explanation of the differences existing between their analyses and those of the writer.

Dumas and Cahours' methods of analysis were substantially the same as those at present in use, and their analysis is doubtless

correct, but their albumin was obtained by concentrating the solution, and it is not probable that the substance analyzed by them was simply coagulated albumin. Ritthausen's analysis agrees well with the writer's, except in nitrogen. He prepared coagulated albumin, however, by acidifying his solution with nitric acid and boiling; therefore, differences are to be expected.

The albumin separating at 80° was present in only very small quantity and no attempt was made to prepare it for analysis.

b.—The Proteids remaining in Solution after Separating the Albumins.

As already stated in describing the reaction of the extract freed from globulin by dialysis, there was found in it one or more proteoses, besides the albumins just described. These were almost wholly precipitated by saturation with sodium chloride, or by adding 20 per cent. of this salt to the solution together with a little acetic acid.

If the albumins are completely removed by heat and the filtered solution then concentrated, a coagulum gradually develops. A similar coagulum has been found in all the extracts of seeds so far examined by the writer. This substance must be derived from the proteose-like proteid, as this forms nearly, if not quite all, the proteid substance remaining in solution before concentration. If the coagulum is removed by concentration and long-continued heating and subsequent filtration, wholly uncoagulable proteose-like substances are found in solution. It is nearly certain that these are the result of alteration of the proteose first described. The amount of proteose is extremely small and no preparations were made for analysis. The coagula obtained by concentrating the solution filtered from preparations **3** and **4**, respectively, were analyzed with the following result:

COAGULATED WHEAT PROTEID, *Preparation* **6**.

	I.	II.	Average.	Ash-free.
Carbon	51.35	51.60	51.48	51.62
Hydrogen	..	..	..	..
Nitrogen	16.79	16.58	16.69	16.73
Sulphur } Oxygen }	..	..	..	..
Ash	0.27	..	..	..

Ash.—0.2626 gram substance dried at 110° gave 0.0007 gram ash = 0.27 per cent.

Carbon and Hydrogen, I.—0.2262 gram dried substance gave 0.4259 gram CO_2 = 51.35 per cent. C, and hydrogen was lost.

II.—0.1695 gram dried substance gave 0.3207 gram CO_2 = 51.60 per cent. C, and hydrogen was lost.

Nitrogen, I.—0.2510 gram dried substance gave 35.23 cc. N at 11° (barometer 756.4 mm. at 23°) = 16.79 per cent.

II.—0.2873 gram dried substance gave 39.21 cc. N at 11° (barometer 768 mm. at 23°) = 16.58 per cent.

Another coagulum similarly obtained from the solution filtered from preparation **4** was also analyzed and the following figures obtained:

COAGULATED WHEAT PROTEID, *Preparation* **7.**

	I.	II.	Average.	Ash-free.
Carbon	51.73	..	51.73	51.86
Hydrogen	6.82	..	6.82	6.82
Nitrogen	17.29	17.26	17.28	17.32
Sulphur } Oxygen }	..	..	..	24.00
Ash	0.25	..	0.25	..
				100.00

Ash.—0.2820 gram substance dried at 110° gave 0.0007 gram ash = 0.25 per cent.

Carbon and Hydrogen.—0.2482 gram dried substance gave 0.4708 gram CO_2 = 51.73 per cent. C, and 0.1525 gram H_2O = 6.82 per cent. H.

II.—0.2096 gram dried substance gave 0.3930 gram CO_2 = 51.14 per cent. C, and 0.1281 gram H_2O = 6.79 per cent. H.

Nitrogen, I.—0.2643 gram dried substance gave 39.21 cc. N at 17° (barometer 752.6 mm. at 22°) = 17.29 per cent.

II.—0.2585 gram dried substance gave 38.02 cc. N at 17° (barometer 757.2 mm. at 20°) = 17.26 per cent.

III.—PROTEIDS SOLUBLE IN SODIUM-CHLORIDE SOLUTION.

Beside the proteids soluble in water, 10-per cent. sodium-chloride brine extracts from ground wheat kernels a globulin which is present in the seed in small quantity. 10,000 grams of "straight flour" were extracted with 34 liters of 10-per cent. sodium-chloride solution by suspending the flour in the liquid, stirring frequently, and then allowing the whole to stand at rest over night. The extract, separated from the flour and filtered as clear as pos-

sible, had a very slight acid reaction, was of a pink color, very viscid consistence, and formed about one-half of the solution added to the flour. This was saturated with ammonium sulphate, and the resulting precipitate filtered off and dissolved as far as possible in four liters of 10-per cent. sodium-chloride brine. The exceedingly viscid solution so produced was filtered with difficulty. The clear solution, placed in a dialyzer, was left in a stream of running water until the chlorides were removed. As the salts dialyzed out, the globulin gradually separated, and under the microscope the precipitate was seen to consist of exceedingly minute particles, the larger of these being evidently spheroidal in form. This precipitate was filtered off, and, after washing with water, alcohol and ether, found to weigh, dried over sulphuric acid, 5.8 grams. This proteid, dissolved in 10-per cent. sodium-chloride solution, when heated slowly, gave a very slight turbidity at 87°, which increased slightly up to 99°. On boiling, some coagulum developed, and on adding acid to the solution filtered from this coagulum, a very considerable precipitate formed.

Dilution of the solution of the globulin in 10-per cent. sodium-chloride brine precipitated the proteid. Saturation with sodium chloride gave no precipitate. Saturation with magnesium sulphate, and also with ammonium sulphate, completely precipitated the globulin. Dried at 110° this preparation gave the following results when analyzed:

WHEAT GLOBULIN, *Preparation* 8.

	I.	II.	Average.	Ash-free.
Carbon	50.87	50.79	50.83	51.07
Hydrogen	6.74	6.67	6.71	6.75
Nitrogen	18.13	18.25	18.19	18.27
Sulphur	0.97	..	0.97	0.97
Oxygen	..	..	..	22.94
Ash	0.48	..	..	..
				100.00

Ash.—0.6010 gram substance dried at 110° gave 0.0029 gram ash = 0.48 per cent.

Carbon and Hydrogen, I.—0.2724 gram dried substance gave 0.5081 gram CO_2 = 50.87 per cent. C, and 0.1652 gram H_2O = 6.74 per cent. H.

II.—0.3169 gram dried substance gave 0.5902 gram CO_2 = 50.79 per cent. C, and 0.1905 gram H_2O = 6.67 per cent. H.

Nitrogen, I.—0.3530 gram dried substance gave 53.75 cc. N at 11° (barometer 752 mm. at 24°) = 18.13 per cent.

II.—0.2635 gram dried substance gave 39.46 cc. N at 11° (barometer 770.5 mm. at 23°) = 18.25 per cent.

Sulphur.—0.6243 gram dried substance gave 0.0441 gram $BaSO_4$ = 0.97 per cent. S.

Another preparation was made in the same way as the preceding, except the precipitation with ammonium sulphate was omitted, the filtered extract being placed at once in dialysers. Like the preceding solution, this was at first very viscid, but after the removal of the chloride the viscid property was entirely lost. This viscidity can hardly be due to the globulin, for solutions of the precipitated globulin showed no trace of it. The aqueous extract of the flour had no such property, and it is difficult to say to what this was due unless to the presence of gum. After complete removal of the chlorides the solution was filtered from the precipitate, the latter dissolved in 10-per cent. sodium-chloride solution, and the insoluble matter filtered off. The residue so removed consisted chiefly of an "albuminate" derived from the globulin. This "albuminate" was dissolved in $\frac{2}{10}$-per cent. potash solution, filtered clear, and precipitated by neutralization with $\frac{2}{10}$-per cent. hydrochloric acid. The precipitate thrown down was washed with water, alcohol and ether. Dried over sulphuric acid it weighed 2 grams. The solution of the globulin was dialyzed till free from chlorides, the separated globulin filtered off, washed with water, alcohol and ether, and after drying over sulphuric acid weighed 5 grams. The yield in this extraction was therefore 7 grams, or nearly the same as in the preceding. The composition of this preparation is given by the following analysis:

WHEAT GLOBULIN, *Preparation* 9.

	I.	II.	Average.	Ash-free.
Carbon	50.77	50.63	50.70	51.01
Hydrogen	7.03	6.84	6.93	6.97
Nitrogen	18.43	18.32	18.38	18.48
Sulphur	0.71	..	0.71	0.71
Oxygen	..	..	..	22.83
Ash	0.62	..	..	..
				100.00

Ash.—0.3685 gram substance dried at 110° gave 0.0022 gram ash = 0.62 per cent.

Carbon and Hydrogen, I.—0.2850 gram dried substance gave 0.5306 gram CO_2 = 50.77 per cent. C, and 0.1805 gram H_2O = 7.03 per cent. H.

II.—0.3700 gram dried substance gave 0.6869 gram CO_2 = 50.63 per cent. C, and 0.2278 gram H_2O = 6.84 per cent. H.

Nitrogen, I.—0.3263 gram dried substance gave 50.34 cc. N at 13° (barometer 761.2 mm. at 26°) = 18.43 per cent.

II.—0.3528 gram dried substance gave 54.16 cc. N at 13° (barometer 760.4 mm. at 28°) = 18.32 per cent.

Sulphur.—0.5190 gram dried substance gave 0.0268 gram $BaSO_4$ = 0.71 per cent. S.

The globulin contained in the "shorts" from the spring wheat was next extracted by treating 2000 grams with 10-per cent. sodium-chloride solution for three hours, with frequent stirring, and then squeezing out the extract in a screw press. After the suspended starch had settled, the extract was decanted and saturated with ammonium sulphate. The precipitate was dissolved in 10-per cent. sodium-chloride brine, and the solution filtered from the insoluble matter. The clear extract was then dialyzed till free from chlorides, and the precipitated globulin found to consist of well-formed spheroids and masses of confluent spheroids. The globulin was strongly colored, since much coloring matter was extracted from the shorts, rendering the extract a deep red-brown in color. The precipitate was then filtered off, again dissolved in the salt solution and placed in a dialyzer. When free from chlorides the precipitated globulin was filtered off and found to be still much colored. On washing with water it dissolved, and was therefore washed with dilute alcohol and ether. Dried over suphuric acid it weighed 2.22 grams, and had the following composition:

WHEAT GLOBULIN, *Preparation* **10.**

		Ash-free.
Carbon	50.79	51.00
Hydrogen	6.80	6.83
Nitrogen	18.19	18.26
Sulphur	0.66	0.66
Oxygen	..	23.25
Ash	0.40	..
		100.00

Ash.—0.2767 gram substance dried at 110° gave 0.0011 gram ash = 0.40 per cent.

Carbon and Hydrogen.—0.3033 gram dried substance gave 0.5649 gram CO_2 = 50.79 per cent. C, and 0.1857 gram H_2O = 6.80 per cent. H.

Nitrogen.—0.2420 gram dried substance gave 37.03 cc. N at 19° (barometer 769 mm. at 19°) = 18.19 per cent.

Sulphur.—0.5233 gram dried substance gave 0.0252 gram $BaSO_4$ = 0.66 per cent. S.

The filtrate and washings obtained from this preparation after the second precipitation by dialysis were precipitated by adding a few drops of sodium-chloride solution. The precipitate produced, which was nearly free from coloring matter, was filtered off, washed with dilute alcohol, absolute alcohol and ether, and found to weigh, when dried over sulphuric acid, 1.25 grams. When analyzed it gave the following results:

WHEAT GLOBULIN, *Preparation* **11.**

		Ash-free.
Nitrogen	18.56	18.64
Ash	0.47	..

Ash.—0.5325 gram substance dried at 110° gave 0.0025 gram ash = 0.47 per cent.

Nitrogen.—0.4248 gram dried substance gave 67.60 cc. N at 19° (barometer 758 mm. at 23°) = 18.56 per cent.

The solution filtered from this preparation was next heated to boiling, and the coagulum obtained was filtered off, washed with water, alcohol, and ether and dried over sulphuric acid; this substance weighed 1.0 gram, and when analyzed it was found to contain the same amount of nitrogen as the globulin. It was considered to be a coagulated globulin. Its nitrogen content was as follows:

COAGULATED WHEAT GLOBULIN, *Preparation* **12.**

	I.	II.	Average.
Nitrogen	18.15	18.43	18.29

Nitrogen, I.—0.2496 gram dried substance gave 38.52 cc. N at 16.5° (barometer 757.2 mm. at 15.5°) = 18.15 per cent. N.

II.—0.1420 gram dried substance gave 22.25 cc. N at 16° (barometer 756 mm. at 18°) = 18.43 per cent. N.

The total quantity of globulin obtained from the shorts was in this case 4.47 grams, being nearly twice as much as was similarly obtained from a like quantity of the flour.

Summary of Analyses of Wheat Globulin.

	8.	**9.**	**10.**	**11.**	**12.**	Average.
C	51.07	51.01	51.00	..	..	51.03
H	6.75	6.97	6.83	..	..	6 85
N	18.27	18.48	18.26	18.64	18.29	18.39
S	23.91	0.71	0.66	..	..	0.69
O		22.83	23.25	..	..	23.04
	100.00	100.00	100.00			100.00

No earlier analyses of this proteid substance are on record. Weyl[1] was the first to call attention to the presence of globulin in wheat, and says that "besides vegetable vitellin I detected in the 10-per cent. sodium-chloride extract of the pulverized seeds of wheat, peas, oats, white mustard, and sweet almonds, a second globulin substance." This he calls vegetable myosin and gives its coagulation-point as 55°–60°. It is probable that Weyl in some way mistook for a globulin the albumin already described. Later, Weyl and Bischoff[2] state, "On investigating the proteids of wheat one of us found chiefly an albuminous substance which, on account of its resemblance to myosin, was named vegetable myosin. This vegetable myosin must be the mother-substance of the gluten, since in wheat meal, together with it, other nitrogenous substances exist, at the most, only in very small amount." On what experimental evidence this statement rests the writers have been unable to discover, and in view of our experience we are at a loss to understand it.

Martin[3] considers wheat flour to contain a large amount of globulin of the myosin type, coagulating between 55°–60°, precipitated by saturation with sodium chloride and ammonium sulphate. Here again the small quantity of albumin contained in the flour has evidently been mistaken for a large quantity of vegetable myosin. This perhaps is not surprising, as the precipitates obtained by saturating sodium-chloride extracts with ammonium sulphate appear very bulky, and in the absence of an actual determination of the weight of these precipitates misleading conclusions might easily be reached. The only globulin found by the writers in extracts of wheat meal, either winter or spring wheat, is the one just described, which in properties and composition closely resembles those globulins which have been found in other seeds and assigned to the class of vegetable vitellins. The following table gives all these globulins obtained and analyzed by the writers up to the present time:

	Wheat.	Maize.	Hemp-seed.	Castor-bean.	Squash-seed.	Flax-seed.
C	51.03	51.71	51.28	51.31	51.66	51.48
H	6.85	6.85	6.84	6.97	6.89	6.94
N	18.39	18.12	18.84	18.75	18.51	18.60
S	0.69	0.86	0.87	0.76	0.88	0.81
O	23.04	22.46	22.17	22.21	22.06	22.17
	100.00	100.00	100.00	100.00	100.00	100.00

[1] Zeitschrift f. physiol. Chem. 1, p. 72. [2] Ber. d. chem. Ges. 1880, p. 367.
[3] British Med. Jour. 2, p. 104, 1886.

IV.—Proteids Soluble in Dilute Alcohol.

As already stated, the wheat flour was found to yield to dilute alcohol a considerable amount of proteid matter. Treatment of the residue remaining after extracting the flour with 10-per cent. sodium-chloride brine likewise removed a large amount of proteid, as did also extraction of the gluten obtained by washing the dough with water. Extracts were made with alcohol under all these conditions, and the proteid extracted subjected to repeated fractional precipitations, in order to learn whether it was a single proteid body or a mixture.

a.—Direct Extraction with Dilute Alcohol.

5000 grams of the straight flour were extracted with 10 liters of alcohol, 0.90 specific gravity, and allowed to soak over night. The next morning the mixture was stirred, and, after settling, the clear solution poured off. Three liters more of alcohol, 0.90 sp. gr., were then added, and after standing some time the supernatant liquid was decanted. The residue was put in a screw press and squeezed nearly dry. The solution so obtained was designated "Extract 1." The residue was again treated with four liters of 0.90-sp. gr. alcohol and pressed nearly dry. This formed extract 2. The same process twice repeated gave two extracts which, when united, formed extract 3. Each of these three extracts, after filtering perfectly clear, was separately concentrated to one-third its volume, and, after cooling, decanted from the very glutinous, viscid mass which had separated. 1 and 3 yielded much more substance than 2. On stirring with a glass rod the precipitated mass formed a very thick, viscid liquid. This substance was in each case dissolved in a small amount of hot alcohol of 0.90 sp. gr., in which it was very soluble, and the solution was allowed to cool over night. Most of the substance separated on cooling and the liquid was decanted from it. The solution decanted from the second and third extracts was treated with a quantity of distilled water and a little sodium chloride added. This threw down a small precipitate, which on standing collected on the bottom of the vessel as a clear, semi-fluid mass. This was treated with water, absolute alcohol and ether, and yielded preparation **13** from extract 2, and preparation **14** from extract 3. These had the following composition:

WHEAT PROTEID, *Preparation* **13**.

		Ash-free.
Nitrogen	17.05	17.18
Ash	0.76	

Ash.—0.7777 gram substance dried at 110° gave 0.0059 gram ash = 0.76 per cent.

Nitrogen.—0.2675 gram dried substance gave 37.22 cc. N at 6° (barometer = 760.7 mm. at 22°) = 17.05 per cent.

WHEAT PROTEID, *Preparation* **14**.

		Ash-free.
Nitrogen	17.15	17.26
Ash	0.65	

Ash.—0.8767 gram substance dried at 110° gave 0.0057 gram ash = 0.65 per cent.

Nitrogen.—0.3556 gram dried substance gave 49.73 cc. N at 6° (barometer 761.3 mm. at 20°) = 17.15 per cent.

13 weighed 7.27 grams; **14**, 10.70 grams. On examination both were found to contain some fat which could not be wholly removed, as the substance had dried in a dense, horny form. The residues which had separated from the solutions just described were next washed by thoroughly intermixing with distilled water. The water was found to dissolve some of the proteid, which was subsequently precipitated by the addition of a little sodium-chloride solution. After standing over night this precipitate settled to the bottom of the vessel in a transparent layer, from which the solution could be completely decanted. After treating this substance with absolute alcohol it formed a voluminous, white, porous mass. This was digested for some time with ether and the substance dried over sulphuric acid. From extract 1, preparation **15** was obtained, weighing 12.4 grams; from extract 2, united with that from 3, preparation **16**, weighing 8.6 grams.

These preparations were found on analysis to have the following compositions:

WHEAT PROTEID, *Preparation* **15**.

	I.	II.	Average.	Ash-free.
Carbon	52.40	52.58	52.49	52.52
Hydrogen	6.77	6.78	6.78	6.78
Nitrogen	17.52	17.73	17.63	17.64
Sulphur	1.05	1.11	1.08	1.08
Oxygen	..	..	..	21.98
Ash	0.06	..	..	..
				100.00

Ash.—0.5660 gram substance dried at 110° gave 0.0003 gram ash = 0.06 per cent.

Carbon and Hydrogen, I.—0.3830 gram dried substance gave 0.7359 gram CO_2 = 52.40 per cent. C, and 0.2334 gram H_2O = 6.77 per cent. H.

II.—0.3390 gram dried substance gave 0.6536 gram CO_2 = 52.58 per cent. C, and 0.2067 gram H_2O = 6.78 per cent. H.

Nitrogen, I (Kjeldahl method).—0.8799 gram dried substance gave NH_3 = 19.7 cc. HCl sol. (1 cc. HCl = 0.00783 gram N) = 17.52 per cent. N.

II (Dumas' method).—0.2901 gram dried substance gave 42.03 cc. N at 6° (barometer 760.1 mm. at 22°) = 17.73 per cent.

Sulphur, I.—0.5123 gram dried substance gave 0.0391 gram $BaSO_4$ = 1.04 per cent. S.

II.—0.3742 gram dried substance gave 0.0302 gram $BaSO_4$ = 1.11 per cent. S.

WHEAT PROTEID, *Preparation* **16.**

		Ash-free.
Carbon	52.69	52.77
Hydrogen	6.77	6.78
Nitrogen	17.74	17.77
Sulphur	1.26	1.26
Oxygen	..	21.42
Ash	0.15	..
		100.00

Ash.—0.6659 gram substance dried at 110° gave 0.0010 gram ash = 0.15 per cent.

Carbon and Hydrogen.—0.3008 gram dried substance gave 0.5812 gram CO_2 = 52.69 per cent. C, and 0.1833 gram H_2O = 6.77 per cent. H.

Nitrogen.—0.3513 gram dried substance gave 50.34 cc. N at 6° (barometer 769 mm. at 23°) = 17.74 per cent.

Sulphur.—0.6282 gram dried substance gave 0.0577 gram $BaSO_4$ = 1.26 per cent. S.

The residues remaining after washing with distilled water were then digested with alcohol of 0.820 sp. gr., which dissolved much of the substance. After standing some time the strong alcoholic solutions were decanted from the residues and found to consist of milky liquids. The addition of a few drops of 10-per cent. sodium-chloride solution immediately produced in each a very large, curdy precipitate, the liquids from which they separated being left perfectly clear. From the solution from the first extract, which was about one liter in volume, 32.26 grams of substance were obtained, after dehydration with absolute alcohol, digestion with ether and drying over sulphuric acid. This was marked preparation **17.** From the second extract was similarly obtained preparation

18, weighing 5.34 grams, and from the third extract preparation **19**, weighing 17.43 grams. The filtrates from **17** and **18** were found to be almost wholly free from proteid, but that from **19** left on evaporation a residue which, when freed from fat, dehydrated with absolute alcohol and dried over sulphuric acid, weighed 7.53 grams, preparation **20**. These preparations were found to have the following compositions:

WHEAT PROTEID, *Preparation* **17**.

		Ash-free.
Carbon	52.59	52.67
Hydrogen	6.70	6.70
Nitrogen	17.64	17.66
Sulphur	1.22	1.22
Oxygen	..	21.75
Ash	0.15	..
		100.00

Ash.—0.6727 gram substance dried at 110° gave 0.0010 gram ash = 0.15 per cent.

Carbon and Hydrogen.—0.4827 gram dried substance gave 0.9309 gram CO_2 = 52.59 per cent. C, and 0.2913 gram H_2O = 6.70 per cent. H.

Nitrogen.—0.3661 gram dried substance gave 52.85 cc. N at 7° (barometer 761.3 mm. at 20.5°) = 17.64 per cent.

Sulphur.—0.7524 gram dried substance gave 0.0667 gram $BaSO_4$ = 1.22 per cent. S.

WHEAT PROTEID, *Preparation* **18**.

	I.	II.	Average.	Ash-free.
Carbon	52.28	52.54	52.41	52.55
Hydrogen	6.87	6.79	6.83	6.85
Nitrogen	17.90	..	17.90	17.94
Sulphur	1.21	..	1.21	1.21
Oxygen	..	..	..	21.45
Ash	0.27	..	..	..
				100.00

Ash.—0.7722 gram substance dried at 110° gave 0.0021 gram ash = 0.27 per cent.

Carbon and Hydrogen, I.—0.3534 gram dried substance gave 0.6774 gram CO_2 = 52.28 per cent. C, and 0.2187 gram H_2O = 6.87 per cent. H.

II.—0.3290 gram dried substance gave 0.6338 gram CO_2 = 52.54 per cent. C, and 0.2010 gram H_2O = 6.79 per cent. H.

Nitrogen.—0.3922 gram dried substance gave 57.73 cc. N at 7° (barometer 758 mm. at 22°) = 17.90 per cent.

Sulphur.—0.7080 gram dried substance gave 0.0625 gram $BaSO_4$ = 1.21 per cent. S.

Wheat Proteid, *Preparation* 19.

	I.	II.	Average.	Ash-free.
Carbon	52.52	52.82	52.67	52.74
Hydrogen	6.72	6.79	6.76	6.77
Nitrogen	17.60	..	17.60	17.62
Sulphur	1.23	..	1.23	1.23
Oxygen	..	..	..	21.64
Ash	0.14	..	..	..
				100.00

Ash.—0.5696 gram substance dried at 110° gave 0.0008 gram ash = 0.14 per cent.

Carbon and Hydrogen, I.—0.3338 gram dried substance gave 0.6429 gram CO_2 = 52.52 per cent. C, and 0.2020 gram H_2O = 6.72 per cent. H.

II.—0.3135 gram dried substance gave 0.6072 gram CO_2 = 52.82 per cent. C, and 0.1916 gram H_2O = 6.79 per cent. H.

Nitrogen.—0.2980 gram dried substance gave 43.24 cc. N at 5° (barometer 750.7 mm. at 23°) = 17.60 per cent.

Sulphur.—0.7703 gram dried substance gave 0.0687 gram $BaSO_4$ = 1.23 per cent. S.

Wheat Proteid, *Preparation* 20.

	I.	II.	Average.	Ash-free.
Carbon	52.13	52.42	52.28	52.39
Hydrogen	6.97	6.85	6.91	6.93
Nitrogen	17.35	17.19	17.27	17.31
Sulphur	1.35	1.41	1.38	1.38
Oxygen	..	..	..	21.99
Ash	0.22	..	..	..
				100.00

Ash.—0.7550 gram substance dried at 110° gave 0.0017 gram ash = 0.22 per cent.

Carbon and Hydrogen, I.—0.3235 gram dried substance gave 0.6184 gram CO_2 = 52.13 per cent. C, and 0.2030 gram H_2O = 6.97 per cent. H.

II.—0.3679 gram dried substance gave 0.7072 gram CO_2 = 52.42 per cent. C, and 0.2265 gram H_2O = 6.85 per cent. H.

Nitrogen, I.—0.2916 gram dried substance gave 41.42 cc. N at 6° (barometer 757 mm. at 22°) = 17.35 per cent.

II.—0.2703 gram dried substance gave 37.82 cc. N at 6° (barometer 761.3 mm. at 21°) = 17.19 per cent.

Sulphur, I.—0.6960 gram dried substance gave 0.0683 gram $BaSO_4$ = 1.35 per cent. S.

II.—0.6389 gram dried substance gave 0.0655 gram $BaSO_4$ = 1.41 per cent. S.

The residues which remained after treatment with alcohol of 0.820 sp. gr. were then dehydrated with absolute alcohol and digested with ether. After drying over sulphuric acid there was obtained from extract 1, preparation **21**, weighing 63.0 grams; from extract 2, preparation **22**, weighing 2.1 grams; and from extract 3, preparation **23**, weighing 41.2 grams.

WHEAT PROTEID, *Preparation* **21**.

		Ash-free.
Carbon	52.81	52.84
Hydrogen	6.81	6.81
Nitrogen	17.66	17.67
Sulphur	1.11	1.11
Oxygen	..	21.57
Ash	0.06	..
		100.00

Ash.—1.1986 grams substance dried at 110° gave 0.0008 gram ash = 0.06 per cent.

Carbon and Hydrogen.—0.3686 gram dried substance gave 0.7138 gram CO_2 = 52.81 per cent. C, and 0.2260 gram H_2O = 6.81 per cent. H.

Nitrogen.—0.4639 gram dried substance gave 67 cc. N at 7° (barometer 762 mm. at 21°) = 17.66 per cent.

Sulphur.—0.7527 gram dried substance gave 0.0610 gram $BaSO_4$ = 1.11 per cent. S.

WHEAT PROTEID, *Preparation* **22**.

	I.	II.	Average.	Ash-free.
Nitrogen	15.42	15.28	15.35	15.42
Ash	0.42	..	0.42	..

Ash.—0.6362 gram substance dried at 110° gave 0.0027 gram ash = 0.42 per cent.

Nitrogen, I (Kjeldahl method).—0.5380 gram dried substance gave NH_3 = 10.6 cc. HCl sol. (1 cc. = 0.00783 gram N) = 15.42 per cent. N.

Nitrogen, II (Dumas' method).—0.3114 gram dried substance gave 39.21 cc. N at 12° (barometer 769.6 mm. at 21.5°) = 15.28 per cent.

WHEAT PROTEID, *Preparation* **23**.

	I.	II.	Average.	Ash-free.
Carbon	52.90	52.99	52.95	53.02
Hydrogen	6.74	6.82	6.78	6.79
Nitrogen	17.24	17.36	17.30	17.32
Sulphur	1.05	..	1.05	1.05
Oxygen	..	..	..	21.82
Ash	0.15	..	..	..
				100.00

Ash.—0.7720 gram substance dried at 110° gave 0.0012 gram ash = 0.15 per cent.

Carbon and Hydrogen, I.—0.3829 gram dried substance gave 0.7427 gram CO_2 = 52.90 per cent. C, and 0.2325 gram H_2O = 6.74 per cent. H.

II.—0.3433 gram dried substance gave 0.6671 gram CO_2 = 52.99 per cent. C, and 0.2108 gram H_2O = 6.82 per cent. H.

Nitrogen, I (Kjeldahl method).—0.5676 gram dried substance gave NH_3 = 12.5 cc. HCl sol. (1 cc. HCl = 0.00783 gram N) = 17.24 per cent. N.

II (Dumas' method).—0.3571 gram dried substance gave 51.54 cc. N at 6° (barometer 747 mm. at 22°) = 17.36 per cent.

Sulphur.—0.5733 gram dried substance gave 0.0441 gram $BaSO_4$ = 1.05 per cent. S.

Preparation **21**, which constituted the principal fraction of the proteid extracted, was further treated in the following manner: Twenty grams were dissolved in 250 cc. of 0.90-sp. gr. alcohol and found to yield a clear solution, which was then poured into 800 cc. of absolute alcohol, whereby a considerable precipitate was at once separated, leaving the solution milky. This substance was dehydrated with absolute alcohol and digested with ether, yielding preparation **24**. The filtrate was then treated with a few drops of 10 per cent. sodium-chloride solution, causing a heavy precipitate, which on stirring rapidly agglutinated and adhered as a mass to the stirring-rod. This was removed, treated in the usual manner, and marked preparation **25**. The mother-liquor from which this separated, after standing over night, deposited a further small amount of proteid, which, after treatment with absolute alcohol and ether, gave preparation **26**. These three substances were analyzed and found to have the following compositions:

WHEAT PROTEID, *Preparation* **24**.

		Ash-free.
Nitrogen	17.67	17.69
Ash	0.10	..

Ash.—0.6064 gram substance dried at 110° gave 0.0006 gram ash = 0.10 per cent.

Nitrogen.—0.4073 gram dried substance gave 61.10 cc. N at 16° (barometer 756.6 mm. at 15°) = 17.67 per cent.

WHEAT PROTEID, *Preparation* **25**.

	I.	II.	Average.	Ash-free.
Carbon	52.15	52.35	52.25	52.33
Hydrogen	6.93	6.87	6.90	6.91
Nitrogen	17.52	17.84	17.68	17.70
Sulphur, Oxygen	..	..	..	23.06
Ash	0.15	..	..	..
				100.00

Ash.—0.6683 gram substance dried at 110° gave 0.0010 gram ash = 0.15 per cent.

Carbon and Hydrogen, I.—0.3842 gram dried substance gave 0.7347 gram CO_2 = 52.15 per cent. C, and 0.2398 per cent. H_2O = 6.93 per cent. H.

II.—0.3291 gram dried substance gave 0.6316 gram CO_2 = 52.35 per cent. C, and 0.2035 gram H_2O = 6.87 per cent. H.

Nitrogen, I.—0.4639 gram dried substance gave 69.2 cc. N at 16.5° (barometer 756 mm. at 18°) = 17.52 per cent.

II.—0.4538 gram dried substance gave 69.6 cc. N at 17° (barometer 750 mm. at 18°) = 17.84 per cent.

WHEAT PROTEID, *Preparation* **26.**

		Ash-free.
Carbon	52.28	52.38
Hydrogen	7.12	7.13
Nitrogen	17.79	17.82
Sulphur, Oxygen	..	22.67
Ash	0.19	..
		100.00

Ash.—0.6300 gram substance dried at 110° gave 0.0012 gram ash = 0.19 per cent.

Carbon and Hydrogen.—0.3162 gram dried substance gave 0.6061 gram CO_2 = 52.28 per cent. C, and 0.2027 gram H_2O = 7.12 per cent. H.

Nitrogen.—0.3932 gram dried substance gave 60.32 cc. N at 17° (barometer 748.2 mm. at 19°) = 17.79 per cent. N.

If the preceding analyses are brought together as in the following table, the effect of the various fractional solutions and precipitations may be seen at a glance.

Proteid extracted by Direct Treatment of the Flour with Alcohol.

	From 0.9 sp. gr. Alcoholic Solution.		From Water Washings.	
	13.	**14.**	**15.**	**16.**
Carbon	..	..	52.52	52.77
Hydrogen	..	..	6.78	6.78
Nitrogen	17.18	17.26	17.64	17.77
Sulphur	..	..	1.08	1.26
Oxygen	..	..	21.98	21.42
			100.00	100.00
Weight of substance in grams	7.27	10.70	12.40	8.60

	From 0.82 Alcoholic Solution.			From Filtrate from 19.
	17.	18.	19.	20.
Carbon	52.67	52.55	52.74	52.39
Hydrogen	6.70	6.85	6.77	6.93
Nitrogen	17.66	17.94	17.62	17.31
Sulphur	1.22	1.21	1.23	1.38
Oxygen	21.75	21.45	21.64	21.99
	100.00	100.00	100.00	100.00
Weight of substance in grams	32.26	5.34	17.43	7.53

	Residue after Extraction with 0.820 Alcohol.			Fractional Reprecipitations of Preparation 21.		
	21.	22.	23.	24.	25.	26.
Carbon	52.82	..	53.02	..	52.33	52.38
Hydrogen	6.81	..	6.79	..	6.91	7.13
Nitrogen	17.67	15.42	17.32	17.69	17.70	17.82
Sulphur	1.11	..	1.05	..	23.06	22.67
Oxygen	21.57	..	21.82	..		
	100.00		100.00		100.00	100.00
Weight of substance in grams	63.0	2.10	41.20	..	..	..

It is evident that **13** and **14** contain less nitrogen than the great bulk of the proteid extracted. This is due to fat which they were found to contain and which could not be wholly removed by extraction with ether, since in drying these preparations were converted into the horny condition which rendered penetration with ether impossible. By grinding fine and extracting with ether some fat was removed, which proved the correctness of this supposition.

22 was evidently impure, as might be expected, since it contained all of the insoluble particles of the entire extract which had escaped filtration, and owing to its small amount these impurities produced a marked effect on its percentage composition. The same is true of **23**, but as the quantity of proteid is so much greater, this contamination has produced much less effect on its composition. **20** was obtained by evaporating the mother-liquor from **19** nearly to dryness and then extracting with absolute alcohol and ether. It would hardly be expected that under such circumstances it would be absolutely pure. The other preparations are in remarkable agreement, and it is evident that no fractional separation of the extracted proteid has been effected. The

preparations obtained from solution in pure water have the same composition as those from solutions in alcohol of 0.820 sp. gr., and also the same composition as the residue remaining after treatment with each of these reagents. It may safely be concluded that the wheat contains but one proteid soluble in dilute alcohol, especially when the experiments next to be described are considered.

The total amount of proteid contained in these several preparations is 207.83 grams, being equal to 4.16 per cent. of the flour.

b.—Extraction with Dilute Alcohol after extracting the Flour with 10-per cent. Sodium-Chloride Solution.

4000 grams of "straight flour" were extracted with 10-per cent. sodium-chloride brine as long as anything was removed. After squeezing as dry as possible in a screw press, the residue was treated with alcohol so as to yield with the water retained by the meal as nearly as possible a solution containing 75 per cent. of alcohol. After digesting two days with the solvent, the extract was squeezed out in a press and the process repeated three times. Four extracts were thus obtained. These were each concentrated to small volume, cooled, and the solution decanted from the precipitated mass. This was then washed with distilled water. After removing the salts the substance from extracts 1 and 2 dissolved to some extent; that from extract 3 dissolved completely to a turbid solution. By adding sodium chloride the dissolved proteid was precipitated completely.

The residues remaining after washing with water were treated with absolute alcohol, digested with ether and dried over sulphuric acid. The precipitates obtained from the water washings by adding salt were treated in the same way. From extract 1, preparation **27** was obtained, weighing 82.0 grams; from extract 2, preparation **28**, weighing 57.0 grams; from extract 3, after dissolving in water and precipitating with sodium chloride, preparation **29**, weighing 11.3 grams; from extract 4, preparation **30**, weighing only 1.35 grams; and from the united water washings of **27** and **28**, preparation **31**, weighing 5.8 grams. The total weight of these preparations was 157.45 grams, equal to 3.94 per cent. of the flour taken. Their composition is shown by the following analyses.

Wheat Proteid, *Preparation* 27.

	I.	II.	Average.	Ash-free.
Carbon	52.61	..	52.61	52.69
Hydrogen	6.82	..	6.82	6.84
Nitrogen	17.62	17.78	17.70	17.73
Sulphur	1.00	1.04	1.02	1.02
Oxygen	..	..	..	21.72
Ash	0.16	..	..	..
				100.00

Ash.—0.6194 gram substance dried at 110° gave 0.0010 gram ash = 0.16 per cent.

Carbon and Hydrogen.—0.3678 gram dried substance gave 0.7095 gram CO_2 = 52.61 per cent. C, and 0.2259 gram H_2O = 6.82 per cent. H.

Nitrogen, I (Kjeldahl method).—0.8977 gram dried substance gave NH_3 = 20.2 cc. HCl sol. (1 cc. HCl sol. = 0.00783 gram N) = 17.62 per cent. N.

II (Dumas' method).—0.3396 gram dried substance gave 48.72 cc. N at 6° (barometer 769.3 mm. at 21°) = 17.78 per cent.

Sulphur, I.—0.6734 gram dried substance gave 0.0491 gram $BaSO_4$ = 1.00 per cent. S.

II.—0.5685 gram dried substance gave 0.0431 gram $BaSO_4$ = 1.04 per cent. S.

Wheat Proteid, *Preparation* 28.

	I.	II.	Average.	Ash-free.
Carbon	52.65	..	52.65	52.72
Hydrogen	6.85	..	6.85	6.86
Nitrogen	17.87	..	17.87	17.89
Sulphur	0.95	0.94	0.95	0.95
Oxygen	..	..	..	21.58
Ash	0.13	..	..	..
				100.00

Ash.—0.6194 gram substance dried at 110° gave 0.0010 gram ash = 0.16 per cent.

Carbon and Hydrogen.—0.3730 gram dried substance gave 0.7202 gram CO_2 = 52.65 per cent. C, and 0.2301 gram H_2O = 6.85 per cent. H.

Nitrogen.—0.2863 gram dried substance gave 41.83 cc. N at 6° (barometer 759.6 mm. at 21°) = 17.87 per cent.

Sulphur, I.—0.6916 gram dried substance gave 0.0480 gram $BaSO_4$ = 0.95 per cent. S.

II.—0.7947 gram dried substance gave 0.0545 gram $BaSO_4$ = 0.94 per cent. S.

WHEAT PROTEID, *Preparation* 29.

	I.	II.	Average.	Ash-free.
Carbon	52.52	52.67	52.60	52.71
Hydrogen	6.82	6.76	6.79	6.81
Nitrogen	17.72	..	17.72	17.75
Sulphur	1.10	..	1.10	1.10
Oxygen	..	..	..	21 63
Ash	0.21	..	..	..
				100.00

Ash.—0.4231 gram substance dried at 110° gave 0.0009 gram ash = 0.21 per cent.

Carbon and Hydrogen, I.—0.3075 gram dried substance gave 0.5922 gram CO_2 = 52.52 per cent. C, and 0.1887 gram H_2O = 6.82 per cent. H.

II.—0.3338 gram dried substance gave 0.6447 gram CO_2 = 52.67 per cent. C, and 0.2030 gram H_2O = 6.76 per cent. H.

Nitrogen.—0.3296 gram dried substance gave 48.04 cc. N at 14° (barometer 755.2 mm. at 22°) = 17.72 per cent.

Sulphur.—0.7409 gram dried substance gave 0.0596 gram $BaSO_4$ = 1.10 per cent. S.

WHEAT PROTEID, *Preparation* 30.

		Ash-free.
Nitrogen	16.93	17.08
Ash	0.91	..

Ash.—0.3075 gram substance dried at 110° gave 0.0028 gram ash = 0.91 per cent.

Nitrogen (Kjeldahl method).—0.7401 gram dried substance gave NH_3 = 16 cc. HCl sol. (1 cc. HCl sol. = 0.00783 gram N) = 16.93 per cent. N.

WHEAT PROTEID, *Preparation* 31.

		Ash-free.
Carbon	52.62	52.65
Hydrogen	6.83	6.83
Nitrogen	17.78	17.79
Sulphur	1.08	1.08
Oxygen	..	21.65
Ash	0.05	..
		100.00

Ash.—0.7826 gram substance dried at 110° gave 0.0004 gram ash = 0.05 per cent.

Carbon and Hydrogen.—0.3081 gram dried substance gave 0.5945 gram CO_2 = 52.62 per cent. C, and 0.1896 gram H_2O = 6.83 per cent. H.

Nitrogen.—0.3084 gram dried substance gave 45.04 cc. N at 6° (barometer 755.5 mm. at 20.5°) = 17.78 per cent. N.

Sulphur.—0.6815 gram dried substance gave 0.0539 gram $BaSO_4$ = 1.08 per cent. S.

c.—Extraction of Gluten with Dilute Alcohol.

2000 grams of "straight spring wheat flour" were made into a dough with distilled water of 20°, and then washed in a stream of river water of 5°. After washing until nearly all the starch was removed, the gluten was chopped up fine and digested with alcohol of 0.90 sp. gr., at a temperature of about 20°. This extraction was continued with repeatedly renewed portions of alcohol of the same strength, as long as anything was removed. The extracts were united, filtered perfectly clear, and concentrated to about one-fourth their original volume. The residual solution was then cooled and allowed to stand over night to deposit the separated proteid. The supernatant solution was poured off, and the large amount of proteid which had separated was then dehydrated by treatment with absolute alcohol. The decanted mother-liquor from which this proteid had separated, and also the strong alcoholic solution which resulted from dehydrating the precipitated mass, were each precipitated by adding a little sodium-chloride solution. The three products thus obtained were united, digested with fresh quantities of absolute alcohol, in order to complete the dehydration, and then extracted with absolute ether. Dried over sulphuric acid the preparation weighed 82.0 grams, and formed therefore 4.10 per cent. of the flour taken. Dried at 110° this substance had the following composition:

WHEAT PROTEID, *Preparation* **32.**

		Ash-free.
Carbon	52.33	52.58
Hydrogen	6.63	6.67
Nitrogen	17.57	17.65
Sulphur	1.08	1.08
Oxygen	..	22.02
Ash	0.50	..
		100.00

Ash.—0.4222 gram substance dried at 110° gave 0.0021 gram ash = 0.50 per cent.

Carbon and Hydrogen.—0.3697 gram dried substance gave 0.7094 gram CO_2 = 52.33 per cent. C, and 0.2207 gram H_2O = 6.63 per cent. H.

Nitrogen.—0.3193 gram dried substance gave 46.47 cc. N at 11° (barometer 763 mm. at 23°) = 17.57 per cent.

Sulphur.—0.4734 gram dried substance gave 0.0372 gram $BaSO_4$ = 1.08 per cent. S.

Thirty grams of preparation **32** were then dissolved in alcohol of 0.90 sp. gr., and the clear solution evaporated to small volume, cooled, and as no proteid separated, strong alcohol was added until a considerable precipitate resulted, equal to about one-half the dissolved proteid. This precipitate which, if the extracted proteid were a mixture, as stated by Ritthausen, would contain the bulk of the substances insoluble in strong alcohol, was then dehydrated by absolute alcohol and dried over sulphuric acid. It was found to weigh 12 grams. This was marked preparation **33.**

The solution from which this substance had separated must have contained the chief part of the proteid called by Ritthausen gluten fibrin. It was then concentrated to small volume, cooled, water added until a considerable precipitate resulted, the solution then heated until all dissolved, and, after cooling, the mother-liquor was decanted from the separated proteid. This process was repeated four times, and the precipitate finally obtained dehydrated with absolute alcohol, digested with ether, and dried over sulphuric acid. This preparation, **34**, weighed 1.6 grams.

WHEAT PROTEID, *Preparation* **33.**

		Ash-free.
Carbon	52.58	52.68
Hydrogen	6.77	6.78
Nitrogen	17.62	17.65
Sulphur	1.09	1.09
Oxygen	..	21.80
Ash	0.19	..
		100.00

Ash.—0.5204 gram substance dried at 110° gave 0.0010 gram ash = 0.19 per cent.

Carbon and Hydrogen.—0.3339 gram dried substance gave 0.6438 gram CO_2 = 52.58 per cent. C, and 0.2035 gram H_2O = 6.77 per cent. H.

Nitrogen.—0.3509 gram dried substance gave 51.74 cc. N at 11° (barometer 755.6 mm. at 22°) = 17.62 per cent. N.

Sulphur.—0.6358 gram dried substance gave 0.0506 gram $BaSO_4$ = 1.09 per cent. S.

WHEAT PROTEID, *Preparation* **34.**

		Ash-free.
Carbon	52.82	52.84
Hydrogen	7.18	7.18
Nitrogen	17.57	17.57
Sulphur } Oxygen }	..	22.41
Ash	0.04	..
		100.00

Ash.—0.4995 gram substance dried at 110° gave 0.0002 gram ash = 0.04 per cent.

Carbon and Hydrogen.—0.1807 gram dried substance gave 0.3500 gram CO_2 = 52.82 per cent. C, and 0.1168 gram H_2O = 7.18 per cent. H.

Nitrogen.—0.3592 gram dried substance gave 52.25 cc. N at 11° (barometer 763.7 mm. at 25°) = 17.57 per cent.

It is clear from these analyses that no separation into proteids of differing composition had thus been effected.

d.—Extraction of "Shorts" with Dilute Alcohol.

2000 grams of "shorts" from the spring wheat flour were extracted with alcohol of 0.90 sp. gr., and the extract squeezed out with a screw press. The extract, which was a deep red-brown in color, was filtered perfectly clear, and then concentrated by distillation to about one-third. On cooling the proteid separated, leaving the mother-liquor as a deep coffee-brown liquid. This was decanted, the precipitate dissolved in alcohol of 0.90 sp. gr., and again precipitated by concentration and cooling, the strongly colored mother-liquor was decanted and this process repeated. The precipitated proteid was then again dissolved in a little dilute alcohol, and the resulting solution poured into absolute alcohol, thereby precipitating the greater part of the proteid and leaving the alcohol strongly colored. The precipitate was thus freed from a very considerable part of its coloring matter. After digestion with absolute alcohol, and finally with ether, it was dried and analyzed with the following result:

WHEAT PROTEID, *Preparation* 35.

		Ash-free.
Carbon	52.75	53.25
Hydrogen	6.96	7.02
Nitrogen	17.22	17.38
Sulphur	1.36	1.37
Oxygen	..	20.98
Ash	0.95	..
		100.00

Ash.—0.4210 gram substance dried at 110° gave 0.0040 gram ash = 0.95 per cent.

Carbon and Hydrogen.—0.3260 gram dried substance gave 0.6306 gram CO_2 = 52.75 per cent. C, and 0.2043 gram H_2O = 6.96 per cent. H.

Nitrogen.—0.2693 gram dried substance gave 39.21 cc. N at 19° (barometer 768.5 mm. at 21°) = 17.22 per cent.

Sulphur.—0.4224 gram dried substance gave 0.0420 gram $BaSO_4$ = 1.36 per cent. S.

Owing to the fact that this preparation was still contaminated with coloring matter, and also showed slight differences in composition from the proteid extracted by similar treatment from the flour, it was subjected to further treatment with a view to its more complete purification.

A part of the preparation was dissolved in 150 cc. of alcohol of 0.90 sp. gr. and poured into 1000 cc. of absolute alcohol. This produced a turbid liquid, which, on adding a drop or two of sodium-chloride solution, gave a heavy precipitate that rapidly settled, leaving the alcohol colored yellow. This precipitate was again dissolved in diluted alcohol, and precipitated by pouring into ether in order to remove anything soluble in this liquid. No coloring matter was thus removed. The precipitate was then digested with absolute alcohol and dried over sulphuric acid, yielding preparation **36**.

The strong alcoholic solution from which this preparation had separated, on longer standing deposited a small amount of substance which, when dehydrated, yielded preparation **37**. Analysis showed these two substances to have the following composition:

WHEAT PROTEID, *Preparation* **36**.

	I.	II.	Average.	Ash-free.
Carbon	52.57	52.52	52.55	52.85
Hydrogen	6.74	6.80	6.77	6.81
Nitrogen	17.39	..	17.39	17.48
Sulphur } Oxygen }	...	..	..	22.86
Ash	0.54	..	..	..
				100.00

Ash.—0.5215 gram substance dried at 110° gave 0.0028 gram ash = 0.54 per cent.

Carbon and Hydrogen, I.—0.3176 gram dried substance gave 0.6122 gram CO_2 = 52.57 per cent. C, and 0.1924 gram H_2O = 6.74 per cent. H.

II.—0.3263 gram dried substance gave 0.6283 gram CO_2 = 52.52 per cent. C, and 0.1990 gram H_2O = 6.80 per cent. H.

Nitrogen.—0.4450 gram dried substance gave 66.68 cc. N at 17° (barometer 748.5 mm. at 18.5°) = 17.39 per cent. N.

WHEAT PROTEID, *Preparation* **37**.

	I.	II.	Average.	Ash-free.
Carbon	52.43	52.56	52.50	52.74
Hydrogen	6.79	6.88	6.84	6.87
Nitrogen	17.59	..	17.59	17.67
Sulphur } Oxygen }	..	..	..	22.72
Ash	0.46	..	..	..
				100.00

Ash.—0.3467 gram substance dried at 110° gave 0.0016 gram ash = 0.46 per cent.

Carbon and Hydrogen, I.—0.3292 gram dried substance gave 0.6329 gram CO_2 = 52.43 per cent. C, and 0.2013 gram H_2O = 6.79 per cent. H.

II.—0.3159 gram dried substance gave 0.6062 gram CO_2 = 52.56 per cent. C, and 0.1958 gram H_2O = 6.88 per cent. H.

Nitrogen.—0.3426 gram dried substance gave 51.64 cc. N at 16.5° (barometer 751.6 mm. at 18.5°) = 17.59 per cent. N.

These figures show that the proteid extracted from the bran has the same composition as that similarly obtained from the flour.

e.—Proteid extracted by Alcohol from whole Wheat Flour.

As Ritthausen and probably others used whole wheat flour in extracting the various proteids described by them as soluble in dilute alcohol, it was thought best to carry out some extractions with meal obtained by grinding the entire wheat kernel in the laboratory.

1000 grams of whole spring wheat flour, freshly ground, were made into a dough, and the gluten obtained from this by washing with water. The resulting gluten was then chopped fine and thoroughly extracted with alcohol of 0.90 sp. gr., the yellow extract concentrated and the proteid separated by cooling. The deposit thus produced was dissolved as far as possible in dilute alcohol, and the insoluble substance, which was coagulated proteid, was washed with dilute alcohol, absolute alcohol and ether, and dried over sulphuric acid. This was preparation **38**.

The solution filtered from **38** was poured into absolute alcohol and a small amount of proteid separated; this was treated with absolute alcohol and ether in the usual way, yielding preparation **39**. The filtrate from **39** was concentrated to small volume and poured into absolute alcohol, whereby nearly all the proteid was

precipitated. This substance was dehydrated with absolute alcohol and digested with ether, giving preparation **40**. These three bodies had the composition shown by the following figures:

COAGULATED WHEAT PROTEID, *Preparation* **38**.

	I.	II.	Average.	Ash-free.
Carbon	52.88	52.68	52.78	52.90
Hydrogen	7.00	6.97	6.98	6.99
Nitrogen	17.47	17.48	17.48	17.52
Sulphur	1.43	..	1.43	1.43
Oxygen	..	..	..	21.16
Ash	0.23	..	..	..
				100.00

Ash.—0.4323 gram substance dried at 110° gave 0.0010 gram ash = 0.23 per cent.

Carbon and Hydrogen, I.—0.2522 gram dried substance gave 0.4890 gram CO_2 = 52.88 per cent. C, and 0.1587 gram H_2O = 7.00 per cent. H.

II.—0.2802 gram dried substance gave 0.5413 gram CO_2 = 52.68 per cent. C, and 0.1760 gram H_2O = 6.97 per cent. H.

Nitrogen, I.—0.2985 gram dried substance gave 44.24 cc. N at 16° (barometer 757.3 mm. at 17°) = 17.47 per cent.

II.—0.3305 gram dried substance gave 49.33 cc. N at 19° (barometer 760.7 mm. at 20°) = 17.48 per cent.

Sulphur.—0.3560 gram dried substance gave 0.0371 gram $BaSO_4$ = 1.43 per cent. S.

WHEAT PROTEID, *Preparation* **39**.

	I.	II.	Average.	Ash-free.
Carbon	52.59	52.73	52.66	52.89
Hydrogen	6.87	6.80	6.84	6.87
Nitrogen	17.92	18.06	17.99	18.06
Sulphur	0.92	..	0.92	0.92
Oxygen	..	..	..	21.26
Ash	0.45	..	...	..
				100.00

Ash.—0.4190 gram dried substance gave 0.0019 gram ash = 0.45 per cent.

Carbon and Hydrogen, I.—0.3267 gram dried substance gave 0.6300 gram CO_2 = 52.59 per cent. C, and 0.2022 gram H_2O = 6.87 per cent. H.

II.—0.2975 gram dried substance gave 0.5752 gram CO_2 = 52.73 per cent. C, and 0.1820 gram H_2O = 6.80 per cent. H.

Nitrogen, I.—0.3637 gram dried substance gave 56.15 cc. N at 20° (barometer 757.2 mm. at 24°) = 17.92 per cent.

II.—0.4018 gram dried substance gave 61.89 cc. N at 20° (barometer 764.8 mm. at 25°) = 18.06 per cent.

Sulphur.—0.4711 gram dried substance gave 0.0316 gram $BaSO_4$ = 0.92 per cent. S.

WHEAT PROTEID, *Preparation* **40**.

	I.	II.	Average.	Ash-free.
Carbon	51.03	50.93	50.98	53.16
Hydrogen	6.59	6.50	6.55	6.83
Nitrogen	16.98	17.08	17.03	17.75
Sulphur	0.92	..	0.92	0.96
Oxygen	..	..	..	21.30
Ash	4.11	4.03	4.07	..
				100.00

Ash, I.—0.5397 gram substance dried at 110° gave 0.0222 gram ash = 4.11 per cent.

II.—0.4313 gram dried substance gave 0.0174 gram ash = 4.03 per cent.

Carbon and Hydrogen, I.—0.2652 gram dried substance gave 0.4962 gram CO_2 = 51.03 per cent. C, and 0.1574 gram H_2O = 6.59 per cent. H.

II.—0.3378 gram dried substance gave 0.6308 gram CO_2 = 50.93 per cent. C, and 0.1976 gram H_2O = 6.50 per cent. H.

Nitrogen, I.—0.3518 gram dried substance gave 51.34 cc. N at 20° (barometer 758.7 mm. at 22°) = 16.98 per cent.

II.—0.3100 gram dried substance gave 45.23 cc. N at 19° (barometer 761 mm. at 21°) = 17.08 per cent.

Sulphur.—0.3458 gram dried substance gave 0.0233 $BaSO_4$ = 0.92 per cent. S.

In a similar manner an extract was made of winter wheat meal, obtained by grinding the entire wheat kernel in the laboratory, the alcoholic extract cencentrated to about one-third its volume, cooled, and the solution decanted from the deposited proteid. This was then dissolved in alcohol of 0.90 sp. gr. and the coagulated proteid filtered off, washed with dilute alcohol, digested with absolute alcohol and then with ether, giving preparation **41**. The solution filtered from this preparation was concentrated to small volume, cooled, and the proteid which separated was digested with absolute alcohol and with ether, yielding preparation **42**.

COAGULATED WHEAT PROTEID, *Preparation* **41**.

		Ash-free.
Carbon	52.64	52.82
Hydrogen	6.86	6.88
Nitrogen	17.49	17.55
Sulphur } Oxygen }	..	22.75
Ash	0.35	..
		100.00

Ash.—0.4275 gram substance dried at 110° gave 0.0015 gram ash = 0.35 per cent.

Carbon and Hydrogen.—0.3232 gram dried substance gave 0.1995 gram H_2O = 6.86 per cent. H, and 0.6234 gram CO_2 = 52.64 per cent. C.

Nitrogen.—0.3316 gram dried substance gave 48.51 cc. N at 11° (barometer 754.8 mm. at 18°) = 17.49 per cent.

WHEAT PROTEID, *Preparation* **42.**

		Ash-free.
Carbon	52.66	52.68
Hydrogen	6.80	6.81
Nitrogen	17.62	17.63
Sulphur } Oxygen }	..	22.88
Ash	0.04	..
		100.00

Ash.—0.4731 gram substance dried at 110° gave 0.0002 gram ash = 0.04 per cent.

Carbon and Hydrogen.—0.2769 gram dried substance gave 0.5347 gram CO_2 = 52.66 per cent. C, and 0.1686 gram H_2O = 6.80 per cent. H.

Nitrogen.—0.3575 gram dried substance gave 52.17 cc. N at 10° (barometer 760.1 mm. at 22°) = 17.62 per cent.

The complete extraction of this proteid from the gluten is very difficult, a little generally remaining in the insoluble residue after extracting with dilute alcohol.

In one case the residue thus remaining was dissolved in $\frac{2}{10}$-per cent. potash water, and the resulting solution, after standing some time to deposit suspended impurities, was decanted and precipitated with dilute hydrochloric acid. This precipitate was washed by decantation with water and then digested for some time with dilute alcohol. The alcoholic solution was then filtered and concentrated to small volume and cooled. The proteid separated was then digested with absolute alcohol and with ether, and dried at 110° for analysis. From this analysis it is seen that the proteid soluble in dilute alcohol is not changed in composition by solution in potash water, nor is its solubility altered so far as could be learned.

WHEAT PROTEID, *Preparation* **43.**

	I.	II.	Average.	Ash-free.
Carbon	52.03	51.88	51.96	52.24
Hydrogen	6.66	6.69	6.68	6.71
Nitrogen	17.48	..	17.48	17.57
Sulphur	1.08	..	1.08	1.08
Oxygen	..	..	..	22.40
Ash	0.52	..	..	..
				100.00

Ash.—0.4222 gram substance dried at 110° gave 0.0022 gram ash = 0.52 per cent.

Carbon and Hydrogen, I.—0.2763 gram dried substance gave 0.5271 gram CO_2 = 52.03 per cent. C, and 0.1658 gram H_2O = 6.66 per cent. H.

II.—0.3705 gram dried substance gave 0.7048 gram CO_2 = 51.88 per cent. C, and 0.2232 gram H_2O = 6.69 per cent. H.

Nitrogen.—0.3923 gram dried substance gave 57.54 cc. N at 11° (barometer 753 mm. at 23°) = 17.48 per cent.

Sulphur.—0.6614 gram dried substance gave 0.0521 gram $BaSO_4$ = 1.08 per cent. S.

In order to facilitate a comparison of these analyses they have been brought together in the following table:

Proteid obtained from Flour by Direct Extraction with Dilute Alcohol.

	13.[1]	14.[1]	15.	16.	17.	18.	19.
C	..	..	52.52	52.77	52.67	52.55	52.74
H	..	..	6.78	6.78	6.70	6.85	6.77
N	17.18	17.26	17.64	17.77	17.66	17.94	17.62
S	..	..	1.08	1.26	1.22	1.21	1.23
O	..	..	21.98	21.42	21.75	21.45	21.64
			100.00	100.00	100.00	100.00	100.00

	20.	21.	23.	24.	25.	26.
C	52.39	52.84	53.02	..	52.33	52.38
H	6.93	6.81	6.79	..	6.91	7.13
N	17.31	17.67	17.32	17.69	17.70	17.82
S	1.38	1.11	1.05	..	23.06	22.67
O	21.99	21.57	21.82	..		
	100.00	100.00	100.00		100.00	100.00

Proteid obtained from Flour by Treatment with Dilute Alcohol after Extraction with 10-per cent. Sodium-Chloride Solution.

	27.	28.	29.	31.
C	52.69	52.72	52.71	52.65
H	6.84	6.86	6.81	6.83
N	17.73	17.89	17.75	17.79
S	1.02	0.95	1.10	1.08
O	21.72	21.58	21.63	21.65
	100.00	100.00	100.00	100.00

[1]Omitted in average.

Proteid extracted from Gluten by Dilute Alcohol.

	Flour, Spring Wheat.			Whole Spring Wheat Flour.			
	32.	**33.**	**34.**	**38.**	**39.**	**40.**	**43.**
C	52.58	52.68	52.84	52.90	52.89	53.16	52.24
H	6.67	6.78	7.18	6.99	6.87	6.83	6.71
N	17.65	17.65	17.57	17.52	18.06	17.75	17.57
S	1.08	1.09	22.41	1.43	0.92	0.96	1.08
O	22.02	21.80		21.16	21.26	21.30	22.40
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	Winter.	
	41.	**42.**
C	52.82	52.68
H	6.88	6.81
N	17.55	17.63
S	22.75	22.88
O		
	100.00	100.00

Proteid extracted from Wheat Bran by Dilute Alcohol.

	35.	**36.**	**37.**
C	53.25	52.85	52.74
H	7.02	6.81	6.87
N	17.38	17.48	17.67
S	1.37	22.86	22.72
O	20.98		
	100.00	100.00	100.00

Average of the preceding Figures.

Carbon	52.72
Hydrogen	6.86
Nitrogen	17.66
Sulphur	1.14
Oxygen	21.62
	100.00

e.—Properties of Proteid extracted by Dilute Alcohol.

This substance, when dehydrated by absolute alcohol and thoroughly dried over sulphuric acid, forms a snow-white, friable mass which is easily reduced to a powder. If dried when moistened with dilute alcohol or water it is converted into an amorphous substance which resembles, in appearance, pure gelatin. When thoroughly dried in this condition it is rather more brittle

than gelatin. Dried in thin sheets it is perfectly clear and transparent. When treated in the cold with distilled water it becomes sticky and a part dissolves. If the water is warmed more dissolves, and on boiling much goes into solution. These solutions in warm water deposit a part of the dissolved substance on cooling, leaving the remainder dissolved. This solution in pure water is instantly precipitated by adding a very minute amount of sodium chloride.

In absolute alcohol this proteid is entirely insoluble, but dissolves on adding water, the solubility increasing with the addition of water up to a certain point, and then diminishing. The exact degree of solubility has not been determined for various strengths of alcohol, but mixtures of about 70 per cent. of alcohol and 30 per cent. of water dissolve the proteid in almost indefinite amount. From solutions in strong alcohol as well as from those in very weak alcohol the proteid is precipitated by adding a few drops of solution of sodium chloride, the completeness of the precipitation depending on the strength of the alcohol and the amount of salt added. The more the alcohol varies in strength from 70–80 per cent. the more completely is the substance precipitated.

In extremely dilute acids and alkalies this proteid is readily soluble, and is precipitated from such solutions on neutralization apparently unchanged either in properties or composition. With Millon's reagent, nitric acid and the biuret-test, the usual proteid reactions are given.

Dissolved in concentrated hydrochloric acid, a beautiful violet color is produced which develops slowly.

With warm 50-per cent. sulphuric acid a similar color is produced, which is greatly increased in intensity on boiling.

As already stated, Einhof, in 1805, was the first to discover that gluten contained a proteid substance soluble in dilute alcohol, to which the name of gliadin was afterward given by Taddei. Berzelius considered that this gliadin was a mixture of the body found by Einhof in rye with another proteid which he named mucin. Boussingault prepared for analysis the proteid extracted from gluten by diluted alcohol, and gave analyses of it in its impure state as well as purified by solution in acetic acid and precipitation with ammonia. The latter analysis will be given in the table which follows. De Saussure, like Berzelius, considered the substance extracted by alcohol to be a mixture of two proteid bodies which he called glutin and mucin. The latter he appeared to regard as

the same as Berzelius' mucin, yet its method of preparation was such as to render this improbable. This mucin was obtained by extracting the gluten with hot alcohol, adding an equal volume of water, concentrating the resulting solution to one-sixth the volume, cooling, decanting the mother-liquor, again adding water, concentrating and cooling. This process was repeated until no more substance separated on cooling. The resulting solution contained the mucin, which, however, De Saussure did not separate in a pure state. Liebig considered the alcohol-soluble portion of the gluten to be a casein-like compound of a proteid with some unrecognized vegetable acid. He gave to it the name of plant gelatin. He also speaks of mucin in such a way as to indicate his acceptance of De Saussure's statements regarding the existence of this body. He quotes analyses by Jones of the plant gelatin which will be found in the table following. Dumas and Cahours found that on extracting gluten with hot alcohol, a proteid substance was deposited on cooling, which they named plant casein. The concentrated filtrate from this body, on cooling, deposited a proteid which they called glutin. Analyses of both these bodies will be found in the table.

Mulder gave an analysis of "plant gelatin" which he extracted from gluten by treatment with alcohol and purification by repeated precipitations from solution in dilute alcohol by concentration and cooling.

Von Bibra, like Dumas and Cahours, considered that alcohol extracted from gluten both plant casein, which separated from a hot extract on cooling, and plant gelatin, which remained in solution. He gave the composition of these proteids as shown by the table and considered them to be isomers. Günsberg found that on boiling gluten with water a considerable amount of proteid matter was dissolved which, on cooling, was deposited. He regarded this as identical with Taddei's gliadin, and endorsed the latter's view that but two proteids were contained in gluten. Dumas' plant-casein he considered to be a portion of the insoluble part of the gluten which had escaped filtration. His analysis of the proteid extracted from gluten by boiling water, and of that obtained by boiling gliadin with water, are given in the table.

Ritthausen described three separate proteids which were extracted from gluten by dilute alcohol. These he separated from one another by fractional precipitation from alcoholic solutions of

various strengths, and also from solution in acetic acid by varying degrees of neutralization. The proteid soluble in the strongest alcohol he called gluten fibrin; that in less strong alcohol, plant gelatin, and the part soluble in the weakest alcohol, mucedin. Analyses of these products appear in the table. Later investigators have paid no attention to the proteids of wheat soluble in alcohol, with the exception of Sidney Martin, who calls the body thus extracted from gluten "insoluble phytalbumose." He further states that a careful repetition of Ritthausen's work failed to show the presence of any other proteid soluble in alcohol than this phytalbumose, and further, that extraction of the flour with alcohol of 76 to 80 per cent., applied before the addition of water, failed to remove any proteid. This latter statement is in direct contradiction to the experience of the writers, who have never failed to obtain the proteid in this way, and in amounts equal to that extracted from the gluten. As Martin gives no details of his operations, no explanation can be offered.

In the following table is given the analyses of the various investigators just named:

	Boussingault. Gliadin.	Jones. Plant Gelatin.	Dumas and Cahours. Casein.	Dumas and Cahours. Glutin.	Mulder. Plant Gelatin.	Von Bibra. Plant Gelatin.
C	52.3	54.44[1]	53.46	53.27	54.85	53.57
H	6.5	7.42	7.13	7.17	7.05	7.12
N	18.9	15.98	16.04	15.94	15.71	15.57
S	..	..	..	..	0.60	0.88
O	22.3	22.16	23.37	23.62	21.79	22.86
	100.00	100.00	100.00	100.00	100.00	100.00

	Günsberg. Plant Gelatin		Ritthausen. Glutin Fibrin.	Ritthausen. Plant Gelatin.	Ritthausen. Mucedin.
C	52.68	52.65	54.31	52.76	54.11
H	6.77	6.88	7.18	7.10	6.90
N	17.76	17.45	16.89	18.01	16.63
S	..	..	1.01	0.85	0.88
O	22.79	23.02	20.61	21.28[2]	21.48
	100.00	100.00	100.00	100.00	100.00

If Günsberg's and Ritthausen's analyses of plant gelatin are compared with those of the writers, it will be seen that they are in close agreement. The analyses of the predecessors of Günsberg differ very materially, both from each other and those of later

[1] Recalculated for an atomic weight of carbon = 12.
[2] Given by Ritthausen as 21.37.

investigators. This is partly due to the imperfect methods of analysis and partly to insufficient purification of the preparations. As details of the work of these earlier chemists are lacking, a fair criticism of their results cannot be made. It may be said, however, that their analyses are not corroborated by the work done since their publication, and that they are undoubtedly incorrect. Ritthausen's composition of plant gelatin is corroborated in the main by the writers' analyses, and there can no longer be any doubt that it represents nearly the true composition of this proteid. We have, however, been unable to obtain any evidence of the existence of the two remaining proteids, gluten fibrin and mucedin. The numerous attempts to precipitate fractionally the alcohol-soluble proteid, although yielding products soluble in every strength of alcohol, has, in every case where the conditions allowed of preparing the proteid free from fat and other foreign bodies, resulted in giving preparations whose properties and composition were identical. It is our opinion that Ritthausen's gluten fibrin and mucedin are simply impure or altered preparations of his plant gelatin. Sidney Martin holds the same opinion, and Günsberg concluded that gliadin was the only proteid dissolved by alcohol. The mucin of Berzelius and De Saussure must also be considered impure plant gelatin, as we can find no evidence of their existence as definite chemical substances. Dumas and Cahours' casein, Günsberg considers to be a part of the insoluble portion of the gluten which had escaped filtration.

The writers consider this to be a part of the gliadin which, owing to the strength of the alcohol used, was more soluble when *hot* than when cold. We are therefore forced to conclude that in the wheat kernel only one proteid soluble in dilute alcohol exists, and that this is one of the constituents of the gluten. That it occurs in the same form in the gluten as in the seed and that there is no change, so far as this body is concerned, caused by contact of the wheat flour with water, has frequently been suggested during the past few years.

This body was first discovered by Einhof and named by Taddei gliadin on account of its resemblance to glue. Since then it has been named by Liebig plant gelatin, by Dumas and Cahours glutin, and, contaminated with various impurities, it has been called by De Saussure and Berzelius mucin and by Ritthausen gluten fibrin and mucedin. It would appear to the writers there-

fore to be proper that we should return to the original and appropriate name and henceforth call it gliadin.

This proteid is entirely distinct in composition and properties from the alcohol-soluble proteids of the maize and oat kernel.

V.—Proteid insoluble in Water, Saline Solutions, and Alcohol.

As already stated, extraction with the above-named solvents, applied successively, removed but a part of the total proteid contained in the wheat kernel, that remaining being soluble only in dilute acids and alkalies. The following extractions were next made to determine the nature of this body.

a.—Proteid extracted by Dilute Potash Solution after extracting the Flour with 10-per cent. Sodium-Chloride Brine and then with Dilute Alcohol.

After completely extracting from 4000 grams "straight flour" all the proteids soluble in 10-per cent. sodium-chloride solution, the residue was freed wholly from proteids soluble in cold alcohol of 0.90 sp. gr. The resulting residue was then extracted twice with a large quantity of $\frac{1}{10}$-per cent. potash solution. After standing three days at a temperature of 5° with frequent stirring this extract was filtered off and allowed to stand in a cold room until the greater part of the fine starch and other impurities which had escaped filtration had settled. The solution, which was still turbid, was decanted from the sediment and neutralized as exactly as possible with $\frac{2}{10}$-per cent. hydrochloric acid, thereby producing a precipitate which settled rapidly, leaving the filtrate milky in appearance. This precipitate, after decanting the solution, was dissolved in $\frac{2}{10}$-per cent. potash water and set aside to deposit the suspended impurities. After decantation from the sediment which resulted, the solution was filtered, but almost nothing was thus removed. It was then neutralized with $\frac{2}{10}$-per cent. hydrochloric acid and the precipitate produced washed by decantation, first with water and then with dilute alcohol, absolute alcohol and ether. No attempt was made in this case to obtain the whole of the proteid soluble in alkali, as the difficulties presented by slow and imperfect filtration rendered this impossible. 13 grams of substance were obtained which, after drying over sulphuric acid, formed a brownish, horny mass. This, preparation **44**, when dried at 110°, gave the following results on analysis:

WHEAT PROTEID, *Preparation* **44**.

		Ash-free.
Carbon	52.47	52.91
Hydrogen	6.75	6.81
Nitrogen	15.51	15.65
Sulphur	0.86	0.86
Oxygen	..	23.77
Ash	0.88	..
		100.00

Ash.—0.3919 gram substance dried at 110° gave 0.0073 gram ash = 0.88 per cent.

Carbon and Hydrogen.—0.3561 gram dried substance gave 0.6851 gram CO_2 = 52.47 per cent. C, and 0.2163 gram H_2O = 6.75 per cent. H.

Nitrogen.—0.3102 gram dried substance gave 38.82 cc. N at 6° (barometer 769.5 mm. at 22°) = 15.51 per cent.

Sulphur.—0.6108 gram dried substance gave 0.0384 gram $BaSO_4$ = 0.86 per cent. S.

Subsequent preparations of this body led to the idea that it was far from pure, this supposition being supported by the fact that the final solution from which it had been precipitated was turbid. The remainder of the preparation was then dissolved as completely as possible in $\frac{2}{10}$-per cent. potash solution, and by repeated filtration through very dense filter-paper obtained perfectly clear. A considerable insoluble residue remained, which appeared to consist largely of the coagulated form of this proteid. This residue was washed by decantation with water, alcohol and ether, and dried over sulphuric acid and then at 110° C. It was found to contain but 13.68 per cent. of nitrogen, showing that the preparation **44** contained much non-nitrogenous matter. The filtration of the dissolved proteid proceeded very slowly, so that it was deemed best to conduct this operation in a refrigerator at a temperature near 0°. A portion of the filtrate first obtained was removed, precipitated by $\frac{2}{10}$-per cent. hydrochloric acid, washed with water, alcohol and ether, and the preparation, **45**, analyzed with the following results. A second portion similarly yielded preparation **46**.

WHEAT PROTEID, *Preparation* **45**.

	I.	II.	Average.	Ash-free.
Carbon	51.10	..	51.10	52.29
Hydrogen	6.46	..	6.46	6.61
Nitrogen	17.01	..	17.01	17.41
Sulphur	0.91	0.92	0.92	0.94
Oxygen	..	..	..	22.75
Ash	2.28	..	..	..
				100.00

Ash.—0.3233 gram substance dried at 110° gave 0.0074 gram ash = 2.28 per cent.

Carbon and Hydrogen.—0.2468 gram dried substance gave 0.4624 gram CO_2 = 51.10 per cent. C, and 0.1435 gram H_2O = 6.46 per cent. H.

Nitrogen.—0.2439 gram dried substance gave 35.23 cc. N at 16° (barometer 758 mm. at 28°) = 17.01 per cent.

Sulphur, I.—0.3218 gram dried substance gave 0.0213 gram $BaSO_4$ = 0.91 per cent. S.

II.—0.4833 gram dried substance gave 0.0325 gram $BaSO_4$ = 0.92 per cent. S.

Wheat Proteid, *Preparation* 46.

		Ash-free.
Nitrogen	17.17	17.33
Ash	0.97	..

Ash.—0.4214 gram substance dried at 110° gave 0.0041 gram ash = 0.97 per cent.

Nitrogen.—0.3184 gram dried substance gave 46.06 cc. N at 16° (barometer 763.7 mm. at 23°) = 17.17 per cent.

These results show that it is absolutely necessary to filter the solution of this body in potash water *perfectly clear* before the final precipitation, since otherwise a considerable amount of non-nitrogenous matter will be precipitated with it. Another extraction was made by treating 200 grams of "patent flour" from spring wheat with 10-per cent. sodium-chloride solution, added in small quantities so as to make a dough. This dough was then washed with 10-per cent. sodium-chloride brine until nearly all the starch was removed and a gluten obtained similar in all respects to that resulting from treating the flour with water. This gluten was then chopped fine, thoroughly extracted with alcohol of sp. gr. 0.90, and then dissolved at 20° in $\frac{1}{10}$-per cent. potash water. The resulting solution was filtered, but as only a part of the impurities was thus removed, the filtrate was placed in an ice-box in shallow dishes and allowed to deposit a considerable part of the suspended matter. The solution which was decanted from the sediment was only slightly turbid, and was then precipitated by neutralizing with $\frac{2}{10}$-per cent. hydrochloric acid. The precipitate filtered off was washed by decantation with water, extracted thoroughly with dilute alcohol, digested with absolute alcohol and then with ether, and dried over sulphuric acid. This preparation, **47**, was a snow-white, light, porous mass, easily reduced to a powder. It had the following composition:

WHEAT PROTEID, *Preparation* **47.**

	I.	II.	Average.	Ash-free.
Carbon	..	52.18	..	52.50
Hydrogen	..	6.90	..	6.94
Nitrogen	17.11	16.90	17.01	17.22
Sulphur	..	1.00	..	1.00
Oxygen	..	..	..	22.34
Ash	..	0.63	..	..
				100.00

Ash.—0.5626 gram substance dried at 110° gave 0.0038 gram ash = 0.63 per cent.

Carbon and Hydrogen.—0.3050 gram dried substance gave 0.5836 gram CO_2 = 52.18 per cent. C, and 0.1890 gram H_2O = 6.90 per cent. H.

Nitrogen, I.—0.3666 gram dried substance gave 53.15 cc. N at 15° (barometer 756.5 mm. at 22°) = 17.11 per cent.

II.—0.6033 gram dried substance gave 49.33 cc. N at 15° (barometer 759.3 mm. at 21°) = 16.90 per cent.

Sulphur.—0.5449 gram dried substance gave 0.0400 gram $BaSO_4$ = 1.00 per cent. S.

b.—Proteid extracted by Dilute Potash Water after Treatment of the Dough with Water and Extraction with Dilute Alcohol.

2000 grams of "straight flour" from spring wheat were made into a dough with distilled water, and this was washed with river water until the gluten was freed as completely as possible from starch. This gluten was then extracted with 75-per cent. alcohol as long as anything was removed. The insoluble residue was dissolved in $\frac{1.5}{100}$-per cent. potash water and the resulting solution allowed to stand in a cold room for 48 hours. The solution was thus freed from but a part of the suspended matter. After decanting from the sediment the solution was neutralized with dilute hydrochloric acid, the precipitate produced, washed several times by decantation with water, thoroughly extracted with alcohol of 0.90 sp. gr., then with stronger alcohol and finally with absolute alcohol and with ether.

The precipitate was again dissolved in $\frac{1}{10}$-per cent. potash solution and allowed to stand over night. It was then filtered and a part of the clear filtrate first obtained was precipitated by neutralization with $\frac{2}{10}$-per cent. hydrochloric acid. This precipitate was washed with water, alcohol, absolute alcohol and ether, yielding preparation **48**. A part of this preparation **48** was redissolved in

$\frac{2}{10}$-per cent. potash water and found to contain a considerable amount of substance which had become insoluble in consequence of drying. This insoluble portion was filtered off, washed with water, alcohol and ether, and gave preparation **49**. The filtrate from this substance was precipitated with $\frac{2}{10}$-per cent. hydrochloric acid, and the precipitate filtered off and washed with water, alcohol and ether. Through an accident this preparation dried on the filter and could not be removed from the paper. It was then again dissolved in dilute potash water and treated exactly as before, yielding preparation **50**. The following analyses show the composition of these three preparations:

WHEAT PROTEID, *Preparation* **48**.

	I.	II.	Average.	Ash-free.
Carbon	51.59	..	51.59	52.32
Hydrogen	6.72	..	6.72	6.82
Nitrogen	17.34	17.39	17.37	17.61
Sulphur }	..	..	..	23.25
Oxygen }				
Ash	1.40	..	..	..
				100.00

Ash.—0.9136 gram substance dried at 110° gave 0.0128 gram ash = 1.40 per cent.

Carbon and Hydrogen.—0.3983 gram dried substance gave 0.7535 gram CO_2 = 51.59 per cent. C, and 0.2410 gram H_2O = 6.72 per cent. H.

Nitrogen, I.—0.3407 gram dried substance gave 50.14 cc. N at 16° (barometer 757 mm. at 18°) = 17.34 per cent.

II.—0.3542 gram dried substance gave 51.34 cc. N at 13° (barometer 764 mm. at 24°) = 17.39 per cent.

WHEAT PROTEID, *Preparation* **49**.

	I.	II.	Average.	Ash-free.
Carbon	50.79	..	50.79	52.87
Hydrogen	6.62	..	6.62	6.88
Nitrogen	16.38	16.38	16.38	17.05
Sulphur }	..	..	..	23.20
Oxygen }				
Ash	3.94	..	3.94	..
				100.00

Ash.—0.8060 gram substance dried at 110° C. gave 0.0318 gram ash = 3.94 per cent.

Carbon and Hydrogen.—0.3671 gram dried substance gave 0.6836 gram CO_2 = 50.79 per cent. C, and 0.2186 gram H_2O = 6.62 per cent. H.

Nitrogen, I.—0.4013 gram dried substance gave 55.56 cc. N at 15° (barometer 758.4 mm. at 22°) = 16.38 per cent.

II.—0.3166 gram dried substance gave 43.74 cc. N at 13° (barometer 754.4 mm. at 21.5°) = 16.38 per cent.

WHEAT PROTEID, *Preparation* **50.**

	I.	II.	Average.	Ash-free.
Carbon	51.50	51.37	51.44	52.62
Hydrogen	6.70	6.57	6.64	6.80
Nitrogen	16.70	..	16.70	17.08
Sulphur } Oxygen }	..	..	..	23.50
Ash	2.25	..	..	..
				100.00

Ash.—0.4428 gram substance dried at 110° gave 0.0100 gram ash = 2.25 per cent.

Carbon and Hydrogen, I.—0.3955 gram dried substance gave 0.7469 gram CO_2 = 51.50 per cent. C, and 0.2383 gram H_2O = 6.70 per cent. H.

II.—0.3363 gram dried substance gave 0.6335 gram CO_2 = 51.37 per cent. C, and 0.1986 gram H_2O = 6.57 per cent. H.

Nitrogen.—0.3918 gram dried substance gave 54.36 cc. N at 13° (barometer 766.5 mm. at 24°) = 16.70 per cent.

Another lot of gluten made from 1000 grams of "straight flour" was treated in exactly the same way as that last described. This gluten was thoroughly extracted with alcohol of 0.90 sp. gr. and the residue dissolved in about 500 cc. of $\frac{2}{10}$-per cent. potash water. After standing over night the very turbid liquid was decanted from the sediment which had formed, and treated with very dilute acetic acid added to slightly acid reaction. The precipitate produced was washed with water, alcohol, and ether and dissolved again in $\frac{2}{10}$-per cent. potash water. The resulting solution, filtered perfectly clear, was precipitated with $\frac{2}{10}$-per cent. hydrochloric acid, washed by decantation with water, then with dilute alcohol, increased gradually in strength up to absolute alcohol, and finally with ether. Dried over sulphuric acid, a pure white, light mass was obtained. This, preparation **51**, was analyzed with the following results:

WHEAT PROTEID, *Preparation* **51.**

	I.	II.	Average.	Ash-free.
Carbon	52.07	52.23	52.15	52.54
Hydrogen	6.71	6.88	6.80	6.85
Nitrogen	17.23	17.42	17.33	17.46
Sulphur	1.07	..	1.07	1.07
Oxygen	..	..	..	22.08
Ash	0.74	0.76	0.75	..
				100.00

Ash, I.—0.8215 gram substance dried at 110° gave 0.0061 gram ash = 0.74 per cent.

II.—0.3008 gram dried substance gave 0.0023 gram ash = 0.76 per cent.

Carbon and Hydrogen, I.—0.3254 gram dried substance gave 0.6213 gram CO_2 = 52.07 per cent. C, and 0.1963 gram H_2O = 6.70 per cent. H.

II.—0 1690 gram dried substance gave 0.3237 gram CO_2 = 52.23 per cent. C, and 0.1048 gram H_2O = 6.88 per cent. H.

Nitrogen, I.—0.3715 gram dried substance gave 53.15 cc. N at 12° (barometer 764 mm. at 20°) = 17.23 per cent.

II.—0.2906 gram dried substance gave 42 23 cc. N at 11° (barometer 757.3 mm. at 20°) = 17.42 per cent.

Sulphur.—0.3307 gram dried substance gave 0.0258 gram $BaSO_4$ = 1.07 per cent. S.

Again gluten was prepared in the usual manner and extracted with alcohol until everything soluble in that reagent was removed. The residue was then dissolved in $\frac{2}{10}$-per cent. potash water and, in order to carry the operation to an end as rapidly as possible, the solution was at once thrown into a filter. As soon as the filtrate ceased to pass through turbid it was returned to the funnel and the filtration continued in an ice-chest. 20 hours after the first solution of the substance in potash water a considerable proportion of the solution was obtained perfectly clear. This was then precipitated with $\frac{2}{10}$-per cent. hydrochloric acid and treated in the usual manner, giving preparation **52**.

The filtration of the potash solution was continued in the ice-chest and after three days the rest of the solution was obtained clear. This was precipitated and treated in the same manner as the preceding, yielding preparation **53**. In this way it was hoped to learn the effect on this substance of prolonged solution in dilute potash water. The composition of these proteids is shown by the following analyses:

WHEAT PROTEID, *Preparation* **52**.

		Ash-free.
Carbon	52.07	52.38
Hydrogen	6.77	6.81
Nitrogen	17.49	17.59
Sulphur	1.24	1.24
Oxygen	..	21.98
Ash	0.61	..
		100.00

Ash.—0.3429 gram substance dried at 110° gave 0.0021 gram ash = 0.61 per cent.

Carbon and Hydrogen.—0.3781 gram dried substance gave 0.7220 gram CO_2 = 52.07 per cent. C, and 0.2306 gram H_2O = 6.77 per cent. H.

Nitrogen.—0.2616 gram dried substance gave 38.22 cc. N at 14° (barometer 765 mm. at 24°) = 17.49 per cent.

Sulphur.—0.1545 gram dried substance gave 0.0140 gram $BaSO_4$ = 1.24 per cent. S.

WHEAT PROTEID, *Preparation* **53**.

Nitrogen	17.10

Nitrogen.—0.1569 gram dried substance gave 22.65 cc. N at 15° (barometer 758.6 mm. at 21°) = 17.10 per cent.

From those figures it is evident that but little loss of nitrogen is caused by prolonging the solution in dilute potash water.

In order to make sure that this conclusion was correct the experiment was repeated in the same way. During the three days in which the second part of the potash solution was in the ice-box the ice melted and at the conclusion the temperature reached 20°. The two preparations thus obtained, numbered respectively **54** and **55**, contained the following amounts of nitrogen:

WHEAT PROTEID, *Preparation* **54**.

		Ash-free.
Nitrogen	17.32	17.53
Ash	1.23	..

Ash.—0.3654 gram substance dried at 110° gave 0.0045 gram ash = 1.23 per cent.

Nitrogen.—0.2107 gram dried substance gave 30.42 cc. N at 14° (barometer 765.3 mm. at 25°) = 17.32 per cent.

WHEAT PROTEID, *Preparation* **55**.

		Ash-free.
Nitrogen	17.50	17.53
Ash	0.16	..

Ash.—0.4200 gram substance dried at 110° gave 0.0007 gram ash = 0.16 per cent.

Nitrogen.—0.2200 gram dried substance gave 32.12 cc. N at 15° (barometer 764.8 mm. at 21°) = 17.50 per cent.

Another preparation of this substance was made by completely extracting 200 grams of "patent flour" (from spring wheat) with large quantities of alcohol of 0.90 sp. gr. The flour was then

washed with absolute alcohol and air-dried. The dry material was then powdered and made into a dough with distilled water. The resulting dough had considerable coherence, showing that the proteid insoluble in alcohol played an important part in its formation. This dough was then washed on a fine hair-sieve under a stream of running water, but no coherent gluten resulted. The washings were then allowed to settle and the sediment, after decanting the solution, was treated with $\frac{2}{10}$-per cent. potash. The solution so obtained after standing over night was decanted from the sediment and precipitated with $\frac{2}{10}$-per cent. hydrochloric acid and the separated proteid allowed to settle. The solution was then decanted, the precipitate dissolved in $\frac{2}{10}$-per cent. potash water and filtered clear in the ice-box. The filtered solution was then precipitated and the separated substance treated in the usual manner. This preparation, **56**, contained the following amount of nitrogen:

WHEAT PROTEID, *Preparation* **56**.

	I.	II.	Average.	Ash-free.
Nitrogen	17.04	16.93	16.99	17.20
Ash	1.22	..	..	..

Ash.—0.5740 gram substance dried at 110° gave 0.0070 gram ash = 1.22 per cent.

Nitrogen, I.—0.3544 gram dried substance gave 50.74 cc. N at 16° (barometer 765.2 mm. at 22°) = 17.04 per cent.

II.—0.3600 gram dried substance gave 51.34 cc. N at 17° (barometer 766.2 mm. at 21°) = 16.93 per cent.

In order to learn whether any change in the proteids occurred in consequence of contact with aqueous solutions before extracting with potash water, the following experiment was made.

1000 grams of "straight flour" from spring wheat were repeatedly extracted with alcohol of 0.90 sp. gr., and after removing everything soluble in that liquid the alcohol was squeezed out as completely as possible in a screw press and the residue extracted with $\frac{2}{10}$-per cent. potash water. It was found impossible to separate the solution from the undissolved portion of the meal either by filtration or subsidence, on account of the presence of gummy matter. An equal volume of alcohol of sp. gr. 0.820 was therefore added, and on long standing the insoluble substance gradually settled, leaving a comparatively clear, yellow solution. This was then syphoned off and filtered. On account of its

gummy character it filtered very slowly. The clear solution finally obtained was then precipitated with $\frac{2}{10}$-per cent. hydrochloric acid, the precipitate filtered off and dissolved in $\frac{2}{10}$-per cent. potash water. The solution so obtained was filtered clear and precipitated with $\frac{2}{10}$-per cent. hydrochloric acid, the resulting precipitate washed by decantation with water, alcohol, at first dilute then gradually increased to absolute, and finally with ether. Preparation **57** was thus obtained, having the following composition:

WHEAT PROTEID, *Preparation* **57**.

		Ash-free.
Carbon	52.14	52.19
Hydrogen	6.91	6.92
Nitrogen	17.54	17.56
Sulphur } Oxygen {	..	23.33
Ash	0.10	..
		100.00

Ash.—0.7996 gram substance dried at 110° gave 0.0008 gram ash = 0.10 per cent.

Carbon and Hydrogen.—0.3426 gram dried substance gave 0.6550 gram CO_2 = 52.14 per cent. C, and 0.2132 gram H_2O = 6.91 per cent. H.

Nitrogen.—0.2671 gram dried substance gave 39.21 cc. N at 15° (barometer 766 mm. at 22°) = 17.54 per cent.

This analysis shows that the same proteid is extracted by potash water from the flour which has not been in contact with water, as was obtained in the other experiments.

c.—Proteid extracted by Dilute Potash Water after Complete Extraction, with Dilute Alcohol, of Gluten from Whole Wheat Flour.

1000 grams of flour made by grinding the *entire* kernel of spring wheat were made into a dough, washed with water till free from starch, and the gluten obtained extracted thoroughly with dilute alcohol. The residue was then dissolved in $\frac{2}{10}$-per cent. potash water, and after the resulting solution had stood for some time it was decanted from the sediment and precipitated by $\frac{2}{10}$-per cent. hydrochloric acid. The precipitate was washed by decantation with water, thoroughly extracted with dilute alcohol and then with absolute alcohol, and finally with ether, and then redissolved in $\frac{2}{10}$-per cent. potash water.

The solution, after filtering perfectly clear, was precipitated and the precipitate treated in the same manner as all the preceding preparations.

When analyzed it was found to have the following composition:

WHEAT PROTEID, *Preparation* 58.

	I.	II.	Average.	Ash-free.
Carbon	51.59	51.67	51.63	52.19
Hydrogen	6.85	6.87	6.86	6.93
Nitrogen	17.21	17.32	17.27	17.45
Sulphur	0.89	..	0.89	0.90
Oxygen	..	..	..	23.43
Ash	1.07	..	..	..
				100.00

Ash.—0.4095 gram substance dried at 110° gave 0.0043 gram ash = 1.07 per cent.

Carbon and Hydrogen, I.—0.2532 gram dried substance gave 0.4790 gram CO_2 = 51.59 per cent. C, and 0.1561 gram H_2O = 6.85 per cent. H.

II.—0.2469 gram dried substance gave 0.4678 gram CO_2 = 51.67 per cent. C, and 0.1517 gram H_2O = 6.87 per cent. H.

Nitrogen, I.—0.3141 gram dried substance gave 46.07 cc. N at 19° (barometer 763.2 mm. at 26°) = 17.21 per cent. N.

II.—0.1350 gram dried substance gave 19.75 cc. N at 19° (barometer 769 mm. at 19°) = 17.32 per cent.

Sulphur.—0.6637 gram dried substance gave 0.0433 gram $BaSO_4$ = 0.89 per cent. S.

The filtrate from this preparation as well as all the others previously described contained a small amount of proteid matter.

This was then treated with a solution of copper sulphate and the small precipitate thereby produced filtered off, washed with water, alcohol and ether, and found to contain the following amount of nitrogen:

WHEAT PROTEID, *Preparation* 59.

		Ash-free.
Nitrogen	13.28	17.45
Ash	23.88	..

Ash.—0.2542 gram substance dried at 110° gave 0.0607 gram ash = 23.88 per cent.

Nitrogen.—0.3849 gram dried substance gave 43.24 cc. N at 19° (barometer 768.2 mm. at 20°) = 13.28 per cent.

In the same way as 58 a preparation of this proteid was made from flour from the *entire* kernel of winter wheat. The composition of this preparation is shown by the accompanying figures.

WHEAT PROTEID, *Preparation* **60.**

	I.	II.	Average.	Ash-free.
Carbon	51.71	..	51.71	52.03[1]
Hydrogen	6.79	..	6.79	6.83
Nitrogen	17.32	17.44	17.38	17.48
Sulphur } Oxygen }	..	..	..	23.66
Ash	0.62	..	0.62	..
				100.00

Ash.—0.4217 gram substance dried at 110° gave 0.0026 gram ash = 0.62 per cent.

Carbon and Hydrogen.—0.2702 gram dried substance gave 0.1652 H_2O = 6.79 per cent. H, and 0.5123 gram CO_2 = 51.71 per cent. C.

Nitrogen, I.—0.3346 gram dried substance gave 48.09 cc. N at 11.5° (barometer 762 mm. at 17.5°) = 17.32 per cent.

II.—0.4140 gram dried substance gave 60.52 cc. N at 11° (barometer 754 mm. at 22°) = 17.44 per cent.

A second portion of the same solution from which preparation **60** had been precipitated was obtained two days later. This was then treated in the same way and yielded preparation **61**, which was found to have the following composition:

WHEAT PROTEID, *Preparation* **61.**

		Ash-free.
Carbon	51.17	52.44
Hydrogen	6.70	6.86
Nitrogen	17.35	17.81
Sulphur } Oxygen }	..	22.89
Ash	2.62	..
		100.00

Ash.—0.3924 gram substance dried at 110° gave 0.0103 gram ash = 2.62 per cent.

Carbon and Hydrogen.—0.3320 gram dried substance gave 0.2019 gram H_2O = 6.70 per cent., and 0.6229 gram CO_2 = 51.17 per cent. C.

Nitrogen.—0.3135 gram dried substance gave 45.17 cc. N at 10° (barometer 757.7 mm. at 21°) = 17.35 per cent.

If we omit preparations **44** and **47** on account of being obtained from unfiltered solutions, **49** as an altered and insoluble product, **50** as having been subjected to an exceedingly prolonged action of an alkaline solution, and **53**, as no correction for ash could be

[1] Trace of unburned carbon left in boat.

made, we will then have in the following table the analyses which in the opinion of the writers most nearly represent the true composition of this proteid.

PROTEID OF THE WHEAT KERNEL SOLUBLE ONLY IN DILUTE ACIDS AND ALKALIES.

	45.	48.	51.	52.	57.	58.	60.	61.
C	52.29	52.32	52.54	52.38	52.19	52.19	52.03[1]	52.44
H	6.61	6.82	6.85	6.81	6.92	6.93	6.83	6.86
N	17.41	17.61	17.46	17.59	17.56	17.45	17.48	17.81
S	0.94	23.25	1.07	1.24	23.33	23.43	23.66	22.89
O	22.75		22.08	21.98				
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	46.	54.	55.	56.	59.
N	17.33	17.53	17.53	17.20	17.45

	Average.
Carbon	52.34
Hydrogen	6.83
Nitrogen	17.49
Sulphur	1.08
Oxygen	22.26
	100.00

Taddei was the first to describe this proteid, which remains after extraction of the gluten with dilute alcohol. He gave it the name of zymom. Berzelius considered Taddei's zymom to be identical with coagulated albumin. Liebig called this substance plant fibrin, and regarded it as the same as blood fibrin. He quoted analyses by Jones and Scherer, which will be found in the table following. Scherer subsequently published another analysis differing somewhat from that given by Liebig. Dumas and Cahours claim priority to Liebig in the use of this name, plant fibrin, one of them having previously used it in his lectures. They prepared their plant fibrin by washing gluten thoroughly with water, extracting this completely with alcohol and with ether, and then digesting the residue with diastase until starch was removed. The composition given by them for this body will be found in the annexed table.

Von Bibra analyzed "plant fibrin" obtained by boiling gluten with dilute alcohol as long as anything was removed. Ritthausen,

[1] Omitted in average.

whose methods the writers have employed in preparing this substance, obtained a number of preparations of this proteid to which he gave the name "gluten casein." He gave the figures to be found in the table as expressing most nearly the true composition of this body. Weyl and Bischoff considered that gluten resulted through the action of some ferment, present in the seed, upon a myosin-like globulin, and that its constituent proteids were consequently not those previously existing in the grain. Martin held the same view. He rejected Ritthausen's name "gluten casein," and designated this substance as "gluten fibrin," a name used by Ritthausen for a body which he described as existing in gluten, soluble in diluted alcohol.

Chittenden and Smith made many preparations of "gluten casein," following Ritthausen's directions. The alkaline solution of the portion of the gluten insoluble in dilute alcohol they did not filter, but allowed to stand at rest for 48 hours, in order to deposit solid particles. The solution, which was then slightly turbid, was precipitated by neutralization with acetic acid. The average of eight analyses is given in the table.

EARLIER ANALYSES OF THE PROTEID SOLUBLE ONLY IN DILUTE ACIDS AND ALKALIES.

	Jones.	Scherer.		Dumas and Cahours.		von Bibra.	Ritthausen.	Chittenden and Smith.
		1.	2.	1.	2.			
C	52.79	54.60	52.34	53.37	53.23	53.57	52.94	52.87
H	7.02	7.45	7.13	7.02	7.01	6.95	7.04	6.99
N	15.59	15.81	15.36	16.00	16.41	15.70	17.14	15.86
S	} 24.61	22.14	25.17	23.64	23.35	1.02	0.96	1.17
O						22.76	21.92	23.11
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

There can be no doubt that the analyses given by these chemists, with the exception of Ritthausen, and Chittenden and Smith, are incorrect, for the substance analyzed by each contained a large part of the impurities of the gluten, since after extraction with alcohol the residue was not dissolved in any solvent and the starch, bran, etc., contained in it filtered off. Ritthausen's figures are the average of two analyses of preparations obtained from solutions in $\frac{2}{10}$-per cent. potash water which had been filtered, one perfectly clear, giving a product containing 17.21 per cent. N; the other very nearly clear and containing 17.08 per cent. N.

From a solution clarified by subsidence, which, however, was still turbid, he obtained a preparation which agrees very well with the analyses of Chittenden and Smith and also with the writers' preparation **44**. To afford a ready comparison these three analyses are here brought together:

	Chittenden and Smith.	Osborne. **44.**	Ritthausen.
C	52.87	52.91	52.66
H	6.99	6.81	6.76
N	15.86	15.65	16.17
S	1.17	1.06	0.86
O	23.11	23.77	23.35
	100.00	100.00	100.00

These figures show plainly that the alkaline solution of the gluten after extraction with cold dilute alcohol contains something which is not removed by subsidence, but requires filtration through very dense paper to effect its separation. In preparing from commercial gluten a large quantity of the proteids contained in it the writer obtained from the ether washings a considerable amount of phytocholesterin and lecethin together with fat. It is probable that all these bodies are held suspended in the solution by aid of the dissolved proteid as an emulsion, and can only be removed by precipitating the first solution in potash water, extracting the precipitate with alcohol and ether, redissolving, and filtering the resulting solution perfectly clear. The first alkaline solution before precipitating and extracting with alcohol and ether cannot be filtered, as it either passes through the paper turbid or does not run through at all.

It is of course unnecessary to consider Berzelius' theory of the identity of this body with coagulated albumin, or Liebig's and Dumas and Cahours', of its identity with blood fibrin. The theories of Weyl and Bischoff and of Martin, however, deserve attention, as their views are at present very generally accepted by physiological chemists. It will not be desirable here to enter into a consideration of these views, as they will be discussed later in connection with gluten and its formation. On the ground of priority and the fact that the relations to animal proteids which gave rise to the various names subsequently applied to this body, have been proved to have no foundation, it would be desirable to return to Taddei's original name and in future call this proteid

zymom. Unfortunately this name is derived from the Greek word ζύμη, a ferment, and as the results of this investigation show that the supposed ferment-changes do not occur in the formation of gluten, this name is undesirable. As this proteid is especially characteristic of gluten, it seems appropriate to call it *glutenin*, a name suggested to us by Professor S. W. Johnson.

d.—Properties of Glutenin.

Characteristic reactions of a proteid body which can be dissolved only in dilute acids or alkalies are necessarily very few in number. In distilled water, preparations obtained as described, after drying over sulphuric acid, were found to yield a very little substance, which could be detected by caustic potash and cupric sulphate. If the water was warmed slightly the reaction was more pronounced. Diluted alcohol also dissolved a minute amount of proteid matter in the cold, and when heated to boiling, a much greater quantity. It is of course questionable whether the minute amount of substance dissolved by water and by alcohol was not due to a trace of gliadin which had not been completely extracted in the process of preparing the glutenin. The fact that the solution in hot alcohol began to precipitate at once on cooling, and that especial care had been taken in all cases completely to extract all gliadin, makes it most probable that the glutenin is slightly soluble in water and in alcohol, especially if these are warmed.

In very dilute alkalies, as $\frac{1}{10}$-per cent. potash solution, and in very dilute acids, as $\frac{2}{10}$-per cent. hydrochloric acid, this substance, after dehydration with absolute alcohol and drying over sulphuric acid, is slowly soluble, with the exception of a greater or less amount of insoluble residue, depending on the circumstances of its preparation. When freshly precipitated and in the hydrated condition it is extremely soluble in the slightest excess of caustic alkali, and in a somewhat greater but still very slight excess of acid. In this condition it is also soluble in the slightest excess of carbonate-of-soda solution or ammonia. Dried over sulphuric acid it dissolves partly in $\frac{5}{10}$-per cent. sodium-carbonate solution.

Dissolved in concentrated hydrochloric acid it gives a solution slightly yellowish at first, but becoming a deep violet color on standing. In sulphuric acid diluted with an equal volume of water the solution is brownish in color after boiling, and remains

clear when diluted. The undiluted solution, on standing, retains its brown color.

A comparison of the analyses of this proteid with those of gliadin shows a very close agreement. As it is well known that many if not most proteids pass readily into conditions in which their solubility is greatly changed without changing their composition to an extent detectable by the most careful analysis, it would seem proper to consider this body as an altered form of gliadin in which its solubility was changed in much the same way as the so-called albuminates derived from globulins. The writer has shown that the albuminates from several of the crystallizable globulins, while insoluble in cold 10-per cent. sodium-chloride solution, are readily soluble at 50°, and separate again on cooling in a crystalline form. In the same way this insoluble proteid of the gluten dissolves to some extent in hot dilute alcohol and separates on cooling. Such changes in solubility do not necessarily imply a "chemical" change, in the sense in which that term is usually employed, but are probably due to what perhaps ought to be more properly designated as a physical change. It is probable that with many proteid substances solution depends on the formation of hydrates which are soluble in the solvent employed, as, for instance, zein, the principal proteid of maize, is wholly insoluble in water and also in absolute alcohol, but if water be present, solution in alcohol at once takes place, the amount of zein dissolved depending, within certain limits, on the quantity of water present.

It is the writer's opinion that the gluten is made up of two forms of the same proteid, one being soluble in cold dilute alcohol and the other not soluble.

The Amount of the Various Proteids contained in the Kernel of Wheat.

1000 grams of fine meal obtained by freshly grinding the entire kernel of spring wheat, and a like quantity of similar flour from winter wheat, were each extracted with 4000 cc. of 10-per cent. sodium-chloride solution, and as much of the extract as possible obtained by filtration, 2500 cc. of the clear extract being obtained from the spring wheat flour and 2600 from the winter wheat flour. As 100 cc. of solution were used for every 25 grams of flour taken, the amount of extract obtained was approximately equal to that from 625 grams of spring wheat flour and 650 grams of

winter wheat flour. These extracts were then dialyzed until all the chlorides were removed, which required five days. The precipitated globulin was then filtered from each, washed with distilled water, alcohol, absolute alcohol, and ether, removed from the filter and dried at 110°. From the spring wheat extract 3 8398 grams were obtained, equal to 0.624 per cent. of the flour, and from the winter wheat 3.9265 grams, equal to 0.625 per cent.

The filtrates from the globulin were then heated to 65° in a water-bath, and after being held at this temperature for some time the coagulum was filtered off and washed with hot water, alcohol, and ether, removed from the paper and dried at 110°. From the spring wheat 1.9714 grams were obtained, being 0.315 per cent. of the flour, and from the winter wheat 1.9614 grams, equal to 0.302 per cent.

The solutions filtered from each of these coagula were next heated just to boiling and the resulting coagulum filtered off, washed thoroughly, and treated as the preceding preparations had been. The spring wheat extract thus yielded 0.4743 gram, equal to 0.076 per cent.; the winter wheat extract 0.3680 gram, equal to 0.057 per cent.

The two extracts were next concentrated by boiling down over a lamp. They remained clear at first, but when somewhat concentrated, the proteid began to separate as a skin on the surface of the solution. When reduced to about one-fourth its original volume the coagulum was filtered off, washed with boiling water, alcohol, and ether, and dried at 110°. The spring wheat thus yielded 0.8737 gram, equal to 0.139 per cent.; the winter wheat 0.8721 gram, equal to 0.134 per cent. The filtrates from these two preparations were evaporated very nearly to dryness on water-baths. On cooling, much substance separated which, when treated with hot water, dissolved again. The insoluble coagulum was filtered from each, washed and dried in the usual manner. The spring wheat gave 0.8149 gram of substance, being 0.130 per cent. of the flour; the winter wheat 0.5795 gram, being 0.089 per cent. The total amount of proteid coagulating on concentration was therefore 0.269 per cent. for the spring wheat and 0.223 per cent. for the winter wheat.

The filtrates from these second coagula were then again evaporated to a syrup, and as no more insoluble matter separated they were each precipitated by pouring into strong alcohol. Large

precipitates resulted in each case, which, after settling and decantation of the alcohol, were dissolved again in a little water and reprecipitated by pouring into strong alcohol. Much coloring matter and sugar was held in solution, as proved by evaporation of the alcoholic mother-liquors. The precipitates were then thoroughly dehydrated with absolute alcohol, washed with ether and dried at 110°. The spring wheat extract thus yielded 6.9289 grams of substance; the winter wheat, 8.7517 grams. As these preparations were unquestionably very impure, and no practicable method of purification existed which could be carried out without great loss of substance, the nitrogen in each was determined and the proteid calculated by multiplying the result obtained by 6.25. The spring wheat preparation was thus found to contain 3.07 per cent. of nitrogen, equal to 19.19 per cent. of proteid, and the winter wheat preparation 5.15 per cent. nitrogen, equivalent to 32.18 per cent.

The amount of proteose and peptone thus found in the extract from the spring wheat was 1.3297 grams, which equals 0.213 per cent. of the flour, and in the winter wheat extract 2.8063 grams, equal to 0.432 per cent. of the flour.

We thus had in the sodium-chloride solution the following amounts of proteid:

	Spring Wheat.	Winter Wheat.
Globulin	0.624 per cent.	0.625 per cent.
Albumin	0.391	0.359
Coagulum	0.269	0.223
Proteose	0.213	0.432
Total	1.497	1.639

The remainder of the proteid matter of the seed forms the gluten. The proportion of gliadin and glutenin in this gluten was determined in the following manner:

200 grams of spring wheat flour, made from the entire seed, and a like quantity of a similar winter wheat flour, were each made into a dough and thoroughly washed with water as long as starch was removed. The gluten thus obtained, after freeing from loosely adhering moisture, was weighed and exactly one-half dried at 110° to constant weight. The spring wheat was thus found to yield 12.685 per cent. dry gluten, the winter wheat flour 11.858 per cent.

The other half of the gluten was cut up very fine and extracted as thoroughly as possible with alcohol of 0.90 sp. gr. The alcoholic extract was then evaporated to small volume, cooled, and the solution decanted from the precipitated proteid. This precipitate was then extracted with ether and dried at 110°. From the spring wheat gluten 4.3379 per cent. was obtained, and from the winter wheat 4.2454 per cent.

The residue extracted with alcohol was then dried at 110° and weighed. The spring wheat gluten contained 7.80 per cent. insoluble in alcohol, the winter wheat 7.504 per cent. reckoned on the wheat. Nitrogen was then determined in these residues dried at 110°, as well as in the dried gluten and also in the original flours. The washings of the glutens were collected in jars and allowed to settle; the sediments washed with water and with very strong alcohol and dried and weighed. The nitrogen in each was then determined. The results of these determinations and deductions drawn from them are given in the following table:

	Spring.	Winter.
Total nitrogen in the flour	1.950 per cent.	1.940 per cent.
Total gluten in the flour	12.685	11.858
Part of gluten insol. in alcohol	7.800	7.504
Per cent. of nitrogen in gluten	12.010	12.000
Total nitrogen in gluten in per cent. of flour	1.5222	1.4230
Total N in residue of gluten insoluble in alcohol	0.8245	0.7346
Total N extracted by alcohol	0.6977	0.6884
Gliadin (N × 5.68, assuming 17.60 per cent. N in gliadin	3.9630	3.9100
Gliadin by direct weighing	4.3379	4.2454
N in sediment from washing gluten	0.2239	0.1552

	Spring Wheat. N.	Proteid.	Winter Wheat. N.	Proteid.
Glutenin	0.8245 × 5.68 =	4.683	0.7346 × 5.68 =	4.173
Gliadin	0.6977 × 5.68 =	3.963	0.6884 × 5.68 =	3.910
Globulin	0.1148 =	0.624	0.1148 =	0.625
Albumin	0.0657 =	0.391	0.0603 =	0.359
Coagulum	0.0453 =	0.269	0.0379 =	0.223
Proteose	0.0341 =	0.213	0.0791 =	0.432
From H_2O washings of gluten	0.2239 × 5.68 =	1.272	0.1552 × 5.68 =	0.881
Total	2.0050	11.415	1.8703	10.603
Flour	2.10 × 5.68	11.93	1.94 × 5.68	10.96

VII.—The Formation of Gluten.

Wheat, so far as known, is the only plant whose seeds contain proteid matter separable in a coherent form from the other constituents by washing with water. When ground fine and mixed with a suitable quantity of water it yields a dough from which a light, porous bread can be made. The importance of this fact in bread-making is so great that considerable attention has been paid to gluten by the chemists who have studied wheat proteids.

The investigations of Günsberg, Martin, and of the writers, disprove the existence of gluten fibrin and mucedin, which are currently stated to exist in gluten, and demonstrate that, as Taddei maintained, gluten consists of two proteids only.

We have already referred, page 415, to the statements of Weyl and Bischoff that the proteid matter of the wheat kernel is *chiefly* a globulin substance, and that in contact with water it undergoes a change, presumably through the influence of a ferment by which gluten results.

If we examine the statements of these investigators we will find no evidence to support their view. They say: "On investigating the proteids of wheat meal, one of us found principally a globulin substance, which he designated, in consequence of its similarity to myosin of the muscle, vegetable myosin. This vegetable myosin must be the mother-substance of the gluten, since in the wheat meal, together with it, other proteids, if at all, exist only in very small amount." What the reasons were for concluding that the "myosin" formed nearly if not all the proteid of the wheat kernel does not appear. In view of the results obtained by the writers, this statement is certainly erroneous. Direct treatment of the meal with alcohol yielded extracts containing gliadin in exactly the same amount as obtained from the gluten made from an equal quantity of flour, and extraction of either flour or gluten with alcohol, after complete exhaustion with sodium chloride solution, also gave the same proportion of gliadin. This substance must therefore have existed in the seed, having the same composition and properties as in the gluten, and as it forms one-half of the gluten, it leaves the other half only as possibly derived from a globulin body through the influence of a ferment. If Weyl and Bischoff's view were correct, treatment of the flour with 10-per cent. salt solution ought to alter the character and quantity

of the gluten obtained, if not altogether to prevent its formation. This is not so, for the usual amount of gluten can readily be obtained from flour made into dough with 10-per cent. sodium-chloride solution and then washed with the same until starch is removed.

Weyl and Bischoff next state that, "with the aid of a 15-per cent. rock-salt solution the flour was extracted until no proteid could be detected in the extract; the residue of the meal kneaded with water then gave no gluten. *If the globulin substance is extracted no formation of gluten takes place.*" We have found that this is true if the flour is stirred up with a large quantity of salt solution, extracted repeatedly with fresh quantities of the same solution until no more proteid is dissolved, and the excess of solution removed by allowing the residue to drain on a filter as completely as possible. If, however, wheat flour is mixed at first with just sufficient salt solution to make a firm dough, this dough may then be washed indefinitely with salt solution, and will yield gluten as well and as much as if washed with water alone. This difference is due to the fact that when large quantities of salt solution are applied at once the flour fails to unite to a coherent mass and cannot afterwards be brought together, as is possible when treated with smaller quantities of solution.

Weyl and Bischoff then compare the formation of gluten to that of blood fibrin from fibrinogen under the influence of a ferment. They say that the formation of gluten is affected by all the conditions which interfere with the activity of ferments in general. "Large amounts of salt hinder the formation of gluten. Sulphates of magnesium and sodium behave like common salt." These statements are explained by what has been said above.

They then tried unsuccessfully to obtain the ferment in the following manner:

"We allowed meal with an equal weight of 90-per cent. alcohol to stand in closed vessels different lengths of time (in one case four months, then several times 3 to 4 weeks, frequently only 8 to 10 days). The vessels were repeatedly shaken and the yellow-colored alcoholic extract was poured off. The residue was freed from alcohol by pressing and evaporating at the ordinary temperature. After it was stirred up with water, little or no gluten was obtained. Evidently the globulin substance had been coagulated for the most part by the alcohol." It is clear that if the flour

were thus treated, the greater part of the gliadin would be removed. The writer has found that if flour is extracted with dilute alcohol until the gliadin is removed, and the residue freed from alcohol by exposure to the air, the latter will then yield no gluten when treated with water.

Sidney Martin next advanced a somewhat similar theory of the formation of gluten from the proteids contained in the seed. He states that alcohol extracts from gluten but one proteid substance; that this is soluble in hot water but not in cold, and he therefore calls it an insoluble phytalbumose.

The residue of the gluten not dissolved by alcohol is uncoagulated proteid, if the alcohol has not been allowed to act too long. This substance he names gluten fibrin. Martin further says that gluten dissolves almost completely in $\frac{2}{10}$-per cent. hydrochloric acid, or $\frac{2}{10}$-per cent. potash solution, leaving a small residue of fat. The solution gives a copious precipitate when neutralized, but the supernatant liquid still contains a quantity of proteid which is the dissolved insoluble albumose. The whole of the gluten fibrin is reprecipitated by neutralization, that is, it is wholly converted into an albuminate.

Martin then asks: "Does flour contain gluten fibrin? Does it contain insoluble phytalbumose?" The first question he says cannot be answered directly. "The second is answered by extracting flour with 76–80 per cent. alcohol. This ought to contain the insoluble albumose if it were present as such in the flour, *but it does not contain it;* it extracts only fat." This statement is certainly erroneous, for the writer has never failed in many experiments thus to extract this substance (gliadin) from the flour, and that too in the same amount and of the same properties and composition as from the gluten.

Martin concludes that insoluble albumose is not present as such in the flour. He then says: "Before proceeding to mention its precursor, it will be well to state that 10-per cent. sodium-chloride solution extracts from flour a large quantity of globulin and of albumose. This globulin is of the myosin type, coagulating between 55° and 60° C., and precipitated by saturation with sodium chloride and ammonium sulphate. Both the globulin and albumose are present in a much smaller quantity in the watery extract of the flour." From this it is evident that Martin has fallen into the same error as Weyl and Bischoff, mistaking the albumin for a myosin-like globulin, and being greatly misled as to its amount.

Continuing, Martin says: "The direction of the evidence is to show that the insoluble albumose is formed from the soluble. Moreover, I think that the globulin is transformed into the gluten fibrin, for I have been able to obtain from the globulin in solution a body having the same reactions as the gluten fibrin." What this evidence is which by its direction shows that the insoluble albumose is derived from the soluble, is not clear, and Martin makes no further statements on this point. That a body should be obtained from the solution containing the globulin which had the same reactions as the "gluten fibrin" is not surprising, for the so-called "albuminates" derived from nearly all globulins have no characteristic reactions, being merely soluble in dilute acids and alkalies, and precipitated by neutralization in the same way as "gluten fibrin." Martin then states his theory of the formation of gluten in the following scheme:

$$\text{Gluten} = \begin{cases} \text{Gluten fibrin} & \text{—precursor, globulin.} \\ \text{Insoluble albumose} & \text{—} \quad \text{“} \quad \text{soluble albumose.} \end{cases}$$

This cannot be a correct representation of the formation of gluten, for it has been shown to be founded on two erroneous observations: first, that alcohol does not extract proteid matter from the flour when applied directly, and second, that at least one-half the proteid matter of the seed is a myosin-like globulin.

The results obtained by the authors and described in this paper have led to the conclusion that no ferment-action is involved in the formation of gluten; that but two proteid substances are contained in the gluten, glutenin and gliadin, and that these exist in the wheat kernel in the same form as in the gluten, except that in the latter they are combined with water in an amount equal to about twice the weight of the dried proteid. The reasons for this opinion are, first, that alcohol extracts the same gliadin in the same amount, whether applied directly to the flour, to the gluten, or to the flour previously extracted with 10-per cent. sodium-chloride solution; second, that $\frac{2}{10}$-per cent. potash extracts glutenin of uniform composition and properties from flour which has been extracted with alcohol or with 10-per cent. sodium-chloride brine and then with alcohol, as it extracts from gluten which has been exhausted with alcohol. Both glutenin and gliadin are necessary for the formation of gluten, as shown by the following experiments.

A portion of flour was washed completely free from gliadin by means of alcohol of 0.90 sp. gr., next with stronger alcohol, finally

with absolute alcohol, and air-dried. The residue was then rubbed up fine until all lumps were removed, and water carefully added and a dough made of the mass. A tolerably coherent dough was thus obtained, but much less elastic and tough than that produced from the untreated flour. This dough was then washed with water on a sieve, using every precaution to obtain a gluten, but none was formed. In another experiment 7.5 grams of very finely ground air-dried gliadin were mixed intimately with 70 grams of fine corn-starch and distilled water added. A plastic dough was thus produced, but it had no toughness. On adding a little 10-per cent. sodium-chloride solution, the dough became at once tough and elastic. This was then washed with great care on a sieve with cold water, a little 10-per cent. salt solution being added from time to time, but in spite of every precaution no gluten was obtained. The following experiment shows that the gliadin used was capable of forming gluten when glutenin was present, and also that salts have a marked influence on the toughness of resulting dough.

Two portions of flour weighing 100 grams each were taken, and after adding 5 grams of gliadin to one, both were made into dough with the same quantity of water. The two doughs presented marked differences; that to which the gliadin had been added was much tougher and more yellow than the other. They were then washed with water as long as starch separated. The gluten was dried superficially by wiping with a cloth and weighed in the moist state. That from 100 grams of flour to which 5 grams of gliadin had been added weighed 44.55 grams; that from 100 grams of flour alone weighed 27.65 grams. The moist glutens were dried at 110° to constant weight, and both yielded the same proportion of dry gluten, *viz.*, 34.6 per cent. The yield of dry gluten was accordingly, in the first case 15.41 grams, and in the second 9.56 grams. The difference, 5.85 grams, shows that the added gliadin was fully recovered in the gluten.

The figures show that these proteids combine with about twice their weight of water in forming gluten. The fact that the added gliadin entered so readily and completely into the formation of gluten indicates that it exists in the seed as such and undergoes no chemical change during extraction and drying.

The properties observed in testing the separated gliadin show how it acts in forming gluten, and explain many of the points observed by others and attributed to a ferment-action.

When treated with distilled water in small amount the fine ground air-dry gliadin at once forms a sticky mass which, on adding more distilled water, dissolves to a turbid solution. If, however, a very little sodium chloride is added to distilled water and this applied to gliadin that has been first moistened with pure water, a very coherent, viscid mass results which adheres to everything it touches and can be drawn out into long threads. If the gliadin is moistened with 10-per cent. sodium-chloride solution and then treated with a larger quantity of this solution, the substance unites to a plastic mass which can be drawn out into sheets and strings, but is not adhesive. From this it is evident why Ritthausen, in washing flours which gave a fluid gluten obtainable only in small quantity and with great difficulty, found that the addition of calcium sulphate to the wash-water rendered the gluten much more coherent and easily obtainable. The gliadin is thus proved to be the binding material which causes the particles of flour to adhere to one another, thus forming a dough. But the gliadin alone is not sufficient to form gluten, for it yields a soft and fluid mass which breaks up entirely on washing with water. The insoluble glutenin is probably essential by affording a nucleus to which the gliadin adheres and from which it is not mechanically carried away by the wash-water.

It might be supposed that this insoluble glutenin which so nearly resembles gliadin in composition results from an alteration of the latter brought about by the action of the mineral or other constituents of the seed or of the water. This is not probable, for the same amount of gliadin is extracted from flour directly by treating it with alcohol of 0.90 sp. gr. as is obtained from the gluten itself, and also the same amount is obtained after extracting the flour completely with 10-per cent. sodium-chloride solution and then with alcohol. The behavior of the gliadin towards 10-per cent. sodium-chloride solution shows why no gluten was obtained by Weyl and Bischoff from flour extracted with this solvent. The gliadin had under these conditions no adhesive qualities and therefore was unable to bind the flour into a coherent mass. If, however, the salt solution is added in small quantities and the flour kneaded and pressed, the particles are brought together and then adhere tenaciously.

VIII.—Summary.

The proteids contained in the wheat kernel are:

I.—A *globulin* belonging to the class of vegetable vitellins, soluble in saline solutions, precipitated therefrom by dilution and also by saturation with magnesium sulphate or ammonium sulphate, but not by saturation with sodium chloride. Partly precipitated by boiling, but not coagulated at temperatures below 100°. The wheat kernel contains between 0.6 and 0.7 per cent. of this globulin. When dried at 110° its composition was found to be as follows:

Wheat Globulin.

Carbon	51.03
Hydrogen	6.85
Nitrogen	18.39
Sulphur	0.69
Oxygen	23.04
	100.00

II.—An *albumin*, coagulating at 52°, which differs from animal albumin in being precipitated on saturating its solutions with sodium chloride or with magnesium sulphate, but not precipitated on completely removing salts by dialysis in distilled water. It was found to form between 0.3 and 0.4 per cent. of the wheat kernel, and to have the following composition when separated from solution in the coagulated form by heating to 60° C.:

Wheat Albumin.

Carbon	53.02
Hydrogen	6.84
Nitrogen	16.80
Sulphur	1.28
Oxygen	22.06
	100.00

III.—A *proteose*, precipitated (after removing the globulin by dialysis, and the albumin by coagulation) by saturating the solution with sodium chloride, or by adding 20 per cent. of sodium chloride and acidifying with acetic acid. This body was not analyzed in its unaltered form. On concentrating its solutions by boiling, a coagulum was gradually developed which formed about 0.3 per cent. of the wheat kernel and had the following composition:

Coagulum.

Carbon	51.86
Hydrogen	6.82
Nitrogen	17.32
Sulphur, Oxygen	24.00
	100.00

The solution filtered from the substance just described still contained a proteose-like body which was not obtainable in a pure state. Its amount could only be roughly estimated by precipitating the concentrated filtrate from the preceding substance with alcohol, and multiplying the nitrogen contained in the precipitate by 6.25. The amount of this proteose was from 0.2 to 0.4 per cent. of the seed. Both these substances are unquestionably derivatives of some other proteid in the seed, presumably the proteose first mentioned.

IV.—*Gliadin*, soluble in dilute alcohol and forming about 4.25 per cent. of the seed. It has the following composition:

Gliadin.

Carbon	52.72
Hydrogen	6.86
Nitrogen	17.66
Sulphur	1.14
Oxygen	21.62
	100.00

This is the proteid called gliadin by Taddei and plant gelatin by Dumas and Cahours. Mixed with impurities or altered to a greater or less extent by the process of separation, it has been described by Ritthausen under the name of gluten fibrin, plant gelatin, or gliadin, and mucedin, and by Martin has been termed insoluble phytalbumose. The mucin of Berzelius and of De Saussure were impure preparations consisting chiefly of this proteid. It is soluble in distilled water to opalescent solutions which are precipitated by adding a little sodium chloride. It is completely insoluble in absolute alcohol, but slightly soluble in 90-per cent. alcohol, and very soluble in 70- to 80-per cent. alcohol, and is precipitated from these solutions on adding either much water or strong alcohol, especially in the presence of much salts; soluble in very dilute acids and alkalies, and precipitated from these solutions by neutral-

ization, unchanged in properties and composition. This proteid is one on which the formation of gluten largely depends.

V.—*Glutenin*, a proteid insoluble in water, saline solutions and dilute alcohol which forms the remainder of the proteids of the wheat kernel, generally about 4 to 4.5 per cent. of the seed. This substance is soluble in dilute acids and alkalies and is precipitated from such solutions by neutralization. Dissolved in $\frac{2}{10}$-per cent. potash water, precipitated by neutralization and, after thorough extraction with alcohol and ether, again dissolved in potash water, the solution filtered *clear*, precipitated by neutralization and dried at 110°, it has the following composition:

Glutenin.

Carbon	52.34
Hydrogen	6.83
Nitrogen	17.49
Sulphur	1.08
Oxygen	22.26
	100.00

Unless prepared as above described, the impurities are not removed and the analyses are discordant. This proteid was first described by Taddei under the name of zymom. Liebig as well as Dumas and Cahours named it plant fibrin. Ritthausen called it gluten casein, Weyl and Bischoff considered it to be an albuminate form of a myosin-like globulin. Martin named it gluten fibrin and likewise considered it to be an albuminate form of a myosin-like globulin.

VI.—Wheat gluten is composed of gliadin and glutenin. Both these proteids are necessary for its formation. The gliadin with water forms a sticky medium, which by the presence of salts is prevented from becoming wholly soluble. This medium binds together the particles of flour, rendering the dough and gluten tough and coherent. The glutenin imparts solidity to the gluten, evidently forming a nucleus to which the gliadin adheres and from which it is consequently not washed away by water. Gliadin and starch mixed in the proportion of 1 : 10 form a dough, but yield no gluten, the gliadin being washed away with the starch. The flour freed from gliadin gives no gluten, there being no binding material to hold the particles together so that they may be brought into a coherent mass.

Soluble salts are also necessary in forming gluten, as in distilled water gliadin is readily soluble. In water containing salts it forms a very viscid, semi-fluid mass, which has great power to bind together the particles of flour. The mineral constituents of the seeds are sufficient to accomplish this purpose, for gluten can be obtained by washing a dough with distilled water.

VI.—No ferment-action occurs in the formation of gluten, for its constituents are found in the flour having the same properties and composition as in the gluten, even under those conditions which would be supposed completely to remove antecedent proteids or to prevent ferment-action. All the phenomena which have been attributed to ferment-action are explained by the properties of the proteids themselves as they exist in the seed and in the gluten.

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ON THE DECOMPOSITION OF THE SALTS OF MONOCHLORACETIC AND MONOBROMACETIC ACIDS.

By J. H. Kastle and B. C. Keiser.

The interesting results obtained in the study of the decomposition of silver chloracetate in aqueous solution[1] led us to undertake a rather more exhaustive study of the decomposition of other salts of this and similarly constituted acids, especially of the salts of those metals whose chlorides and bromides are freely soluble in water, in the hope of being able to arrive at a more complete solution of the problem presented by such decompositions. In the following pages are given the results of this investigation up to the present time, together with such discussion of the hypothesis originally offered by one of us in explanation of the decomposition of silver chloracetate as these fresh data would seem to warrant.

[1] This Journal **14**, 586.

The Salts of Monochloracetic Acid.

Quite a number of the salts of monochloracetic acid have been prepared by different observers from time to time. The difficulties in the way of obtaining the most of these in anything like a desirable condition of purity will, however, doubtless warrant a brief description, together with an account of the method of preparation of those which have been used in this investigation, despite the fact that many of them, if not all, have already been described.

Anhydrous Potassium Chloracetate.—This salt is best prepared by treating the required amount of caustic potash dissolved in absolute alcohol, with an equivalent quantity of chloracetic acid dissolved in the same solvent. The solution of the acid is slowly added to the solution of caustic potash. The mixture is constantly stirred, and kept cool by immersing the vessel containing it in cracked ice. Under these conditions the anhydrous salt is precipitated in the form of a fine, white, crystalline precipitate. During the operation small portions of the mixture are tested from time to time with phenolphthaleïn, and when found to be entirely neutral the salt thus obtained is filtered off and dried as rapidly as possible on filter-paper. When dry the salt has a pearly luster, is soft and soapy to the touch, stable in dry air, and very soluble in water. On analysis it was found to yield numbers which agree fairly well with those demanded by the theory, and repeated determinations of the amount of chlorine in the condition of chloracetic acid and of that present as free chloride have shown it to contain 97–98 per cent. of pure potassium chloracetate.

Anhydrous Sodium Chloracetate.—The method employed to best advantage in the case of this salt is essentially the same as that given in the above for the preparation of the potassium salt. The salt thus obtained by adding an alcoholic solution of monochloracetic acid to one of caustic soda is anhydrous, pearly in luster, soapy to the touch, very soluble in water, and of about the same degree of purity as the potassium salt.

Anhydrous Ammonium Chloracetate.—This salt is best prepared by treating a saturated solution of ammonia in absolute alcohol with an alcoholic solution of the acid, adding the latter to the former gradually, and keeping the vessel containing the mixture immersed in cracked ice. The pure, anhydrous ammonium

salt separates out under these conditions in the form of beautiful, delicate, white needles.

Magnesium Chloracetate, $(CH_2ClCOO)_2Mg + 2H_2O$. — This salt was made by treating the oxide of magnesium with a concentrated aqueous solution of the acid and evaporating under a bell-jar in a current of dry air. The salt is exceedingly soluble both in alcohol and water. The following numbers were obtained on analysis:

	Found.	Calculated.
Mg	9.76	9.71 per cent.

Calcium Chloracetate, $(CH_2ClCOO)_2.Ca + H_2O$. — This salt was obtained by neutralizing an aqueous solution of the acid with pure, precipitated calcium carbonate and evaporating the solution under a bell-glass in a current of dry air. Like the magnesium salt it is exceedingly soluble in water and also in alcohol. It crystallizes in fine, white needles. On analysis it gave the following numbers:

	Found.	Calculated.
Ca	16.36	16.32 per cent.

Anhydrous Strontium Salt.—The strontium salt was prepared by dissolving the carbonate in an aqueous solution of the acid by the aid of heat, filtering, and adding about an equal volume of alcohol. Under these conditions the salt comes down in the form of a beautiful, white, crystalline precipitate. Of all the salts of monochloracetic acid, it and the barium salt are probably the easiest to obtain in good yield and in a condition of purity. On analysis the following numbers were obtained:

	Found.	Calculated.
Sr	31.74	31.87 per cent.

The barium and lead salts were also prepared by well known methods which need no particular description. It may be said, however, in this connection that the barium and strontium salts would be, in consequence of their purity and the ease with which they may be prepared, excellent material for such an investigation as this, were it not for the fact that they are just a little too insoluble in very small quantities of water.

The Rate of Decomposition of Potassium Chloracetate.

At ordinary temperatures, ranging from 20°–25° C., the decomposition of the potassium salt in water into potassium chloride and

glycollic acid is a very slow one, as is shown by the following experiments:

Time, 94 days; quantity of salt in each case, 0.1325 gram.

	I.	II.	III.
Water	0.9 cc.	2.7 cc.	9 cc.
Percentage decomposed	5	2.5	1.75

When heated with water, however, the decomposition proceeds —in some instances at any rate—with great rapidity, as indicated in the following table:

TABLE I.—*Showing the Effect of Water on the Decomposition of Potassium Chloracetate.*

Time, 2 hours; temperature, 100° C.; quantity of salt in each experiment, 0.1325 gram.

No. of Experiment.	Quantity of Water in cc.	Percentage Decomposed.
1	0.09	75 : 74
2	0.18	68 : 68.5
3	0.27	60 : 59.5
4	0.36	55 : 51
5	0.45	47.5 : 47
6	0.54	43 5 : 43
7	0.63	39.5 : 39.5
8	0.72	36 : ...
9	0.81	34 : 34
10	0.9	32 : 32
11	1.8	22 : 23 : 22
12	2.7	... : ...
13	5.4	... : ...
14	9	14.5 : 14
15	27	8.5 : ...
16	90	9.5 : ...
17	180	9.5 : 9
18	270	11 : 10.5
19	360	11.5 : 11.5
20	450	12 : 12.5
21	540	13.5 : ...
22	1080	8.5 : 9

Two methods of course suggest themselves for measuring the amount of this decomposition. Either the quantity of free chloride

may be determined by means of standard solutions of silver nitrate and ammonium sulphocyanide, or the glycollic acid formed as a result of the decomposition may be measured directly by means of standard alkali. The results given in Table I above were obtained by the latter method, using a $\frac{1}{10}$-normal solution of caustic potash, with phenolphthalein as the indicator.

With the smaller quantities of water, from 0.09 cc. to 1.8 cc., the experiments were tried in sealed glass tubes about 75 mm. long and of 8 mm. inside diameter. The experiments requiring larger amounts of water were tried either in tubes or flasks connected with small inverted condensers. The pressure produced in the sealed tubes with the smaller quantities of water seems to exert no influence upon the speed of the reaction. Of the several numbers obtained in the case of Exp. 11, Table I, two, 22 and 23 per cent., were obtained working in the open tube with inverted condenser, and one, 22 per cent., in the sealed tube.

It will be seen in the above table that the results obtained in the decomposition of potassium chloracetate agree fairly well among themselves, especially when the small quantities both of salt and water are considered, and also that every drop of a $\frac{1}{10}$-normal solution corresponds to about 0.5 per cent.

It is, however, to be remarked in this connection that these results were obtained from the same batch of potassium chloracetate, and that a comparison of the results obtained from different batches of the salt shows a greater variation for corresponding states of dilution. This variation shows itself in those experiments only in which the smaller quantities of water have been employed, *viz.*, from 0.09 cc. to 1 cc.; and even here where it is found to be the greatest, the same relative values are found to hold for the decomposition at corresponding states of dilution. The variation is doubtless to be attributed to the fact that it is practically impossible to obtain any two batches of salt of exactly the same grade of purity. It is to be concluded from these experiments that the decomposition of potassium chloracetate in water, like that observed for the corresponding silver salt, suffers a gradual retardation with increasing quantities of the solvent.

The Rate of Decomposition of Sodium Chloracetate.

TABLE II.—*Showing the Effect of Water on the Decomposition of Sodium Chloracetate.*

Time, 2 hours; temperature, 100° C.; quantity of salt in each experiment, 0.1165 gram.

No. of Experiment.	Quantity of Water in cc.	Percentage Decomposed.
1	0.09	65 : 64
2	0.18	57.5 : 57.5
3	0.27	50.5 : 50.5
4	0.36	45.5 : ...
5	0.45	39 : ...
6	0.54	34.5 : 35.5
7	0.63	32.5 : 32.5
8	0.72	30 : ...
9	0.81	28 : 28
10	0.9	26 : 26.5
11	1.8	18 : 18.5
12	9	11 : 10.5
13	90	7.5 : 6.5
14	180	7.5 : 7
15	270	10 : 10
16	360	10 : 10.5
17	450	10 : 10
18	540	11.5 : 12.5
19	1080	8

The results set forth in Table II require no special comment. What has already been observed in the case of those obtained with the potassium salt applies with equal force to these. They very clearly show the retarding influence of water upon the course of the reaction. It is worthy of note, however, in this connection, that the results obtained both with sodium and potassium salts would seem to indicate that the retarding effect of the water reaches a maximum at some dilution between 90 cc. and 1 litre, the exact point not having been determined, and that consequently the rate of decomposition becomes practically constant. The slight variations which are experienced in passing through the several stages of greater dilution are probably to be accounted for partly by reason of the fact that it was found more difficult to control the temperature where such large quantities of water were employed, and partly by reason of the difficulty of getting a sharp end-reaction with the indicator in these conditions of greater dilution.

The Effect of Time upon the Decomposition of Potassium Chloracetate.

In discussing the results obtained in the study of the decomposition of silver chloracetate it was stated that the decomposition in question was a simple, non-reversible reaction, unattended by the formation of secondary products of any kind. So far as we have been able to discover, the same applies to the decomposition of all the salts of this acid thus far studied, and in this connection it may not be out of place to cite the fact that such attempts as have been made on the part of other observers to prepare chloracetic acid from hydrochloric acid and glycollic acid have given only negative results.

In order finally to settle upon the nature of the chemical process here involved, it was thought desirable to determine not only the effect of varying amounts of water upon the course of the reaction, but also the effect of time. This was done with the following results:

TABLE III.—*Showing the Effect of Time upon the Decomposition of Potassium Chloracetate.*

Temperature, 100° C.; quantity of salt in each experiment, 0.1325; quantity of water, 2.7 cc.; $a = 100$ per cent.

No. of Experiment.	Time = T.	Percentage Decomposed = x.	K.
1	60 minutes	8 : 7.5	.0015
2	120 "	15 : 15	.0014
3	180 "	23 : 22	.0014
4	240 "	31 : 31.5	.0015
5	300 "	36 : 37	.0015
6	360 "	40 : 39.5	.0014
7	420 "	44	.0014
8	480 "	47 : ...	.0013
9	540 "	49 : 50	.0013
10	600 "	54.5 : 55	.0013

It will be seen from these results that the decomposition here studied obeys, for the given dilution, the law for simple decompositions as first established by Wilhelmy for the inversion of cane-sugar, and satisfies the mathematical formula for the same:

$$\log \frac{a}{a-x} = KT \cdot 4343.$$

We may also conclude that in the chemical process here being studied we have but one substance affected.

The Decomposition of the Salts of Monochloracetic Acid in Water is independent of the Nature of the Base.

In view of the hypothesis put forward concerning the decomposition of silver chloracetate it became of interest to determine the extent of the decomposition for the various salts of chloracetic acid at the same or corresponding states of dilution. Such salts as have been described above were employed in these experiments for which the following conditions were observed:

Time = 2 hrs. Temperature = 100° C.

Quantities of water: in Series No. I, 0.9 cc. for the salts of univalent metals, and 1.8 cc. for those of bivalent metals; in Series No. II, 2.7 cc. for the salts of univalent metals, and 5.4 cc. for the salts of bivalent metals; in Series No. III, 9 cc. for the salts of univalent metals, and 18 cc. for those of bivalent metals.

Quantity of each of the several salts: the thousandth part of one molecular weight in grams.

The results obtained in these experiments are given below in Table IV.

TABLE IV.—*Showing the Decomposition of several of the Salts of Monochloracetic Acid under the Same Conditions.*

	I.	II.	III.
Potassium Salt	32. per cent.	15. per cent.	10.7 per cent.
Sodium Salt	25.5	16.	10.75
Ammonium Salt	27.	16.25	10.5
Magnesium Salt	24.5	16.	...
Calcium Salt	26.5	15.75	11.75
Strontium Salt	25.5	15.	11.
Barium Salt	26.7	15.25	10.7
Lead Salt	...[1]	...[1]	10.

These results clearly indicate that the decomposition here taking place is independent of the nature of the base. This of course does not hold for those salts the metals of which yield insoluble chlorides, such as silver, mercury in the mercurous condition, etc.

[1] At these dilutions the lead salt was not completely soluble and hence was not determined.

The Velocity of Decomposition of Potassium Chloracetate at Normal, ½-Normal, and $\frac{1}{10}$-Normal States of Dilution.

In order to be able to compare more accurately the velocity of decomposition with the coefficients of affinity of hydrochloric and monochloracetic acids respectively, the endeavor has been to determine the coefficients of the velocity of the decomposition at *normal*, *½-normal* and *$\frac{1}{10}$-normal* states of dilution. These particular states of dilution having been selected for the reason that the coefficients of affinity of the two acids mentioned have been accurately determined under these conditions.

In consequence of the constant removal of some of the salt from the solution by virtue of its decomposition, no particular state of dilution can be preserved for a sufficient length of time to enable the determination to be made directly. To meet this difficulty the following method was resorted to, which, together with the results obtained, is given below:

No. of Experiment.	Quantity of Salt.	Quantity of Water in cc.	Time in Minutes.	Percentage decomposed.	*K*.
1	0.1325 gram	0.53	135	45.	0.0044
2	"	"	150	49.5	0.0045
3	"	"	165	52.	0.0044
4	"	"	180	55.	0.0044
5	"	1.03	240	47.	0.0026
6	"	"	255	48.	0.0026
7	"	"	285	51.5	0.0026

The several values of K are obtained from the data given in the columns marked "Time" and "Percentage decomposed," by means of the formula $\log\frac{a}{a-x} = KT.4343$, to which attention has already been called (see p. 477).

This coefficient of the velocity of the decomposition in passing from twice-normal to normal is thus found to be 0.0044; while that obtained in passing from normal to ½-normal is found to be 0.0026. The mean therefore of these two values $\frac{0.0044+0.0026}{2} =$ 0.0035 must approximate very closely to the true coefficient of velocity for normal dilution. The extra 0.03 cc. of water over and above that required to form a twice-normal and normal solution of the amount of salt taken was added in these and certain other

experiments, for the reason that, while the potassium salt used was anhydrous, a modification of the potassium salt exists containing one and a half molecules of water of crystallization. Hence, in order to do away with any disturbing influence upon the proper dilution which the tendency on the part of the anhydrous salt to form such a hydrate might produce, it was deemed expedient at the time these experiments were tried, to add the extra 0.03 cc., this amount being the nearest measurable quantity to that required by the theory.

The following results furnish the necessary data for the determination of the coefficient of velocity at $\frac{1}{3}$-normal:

No. of Experiment.	Quantity of Salt taken.	Quantity of Water in cc.	Time in Minutes.	Percentage decomposed.	*K*.
1	0.1325 gram	2.28	180	25	0.001597
2	"	3.03	195	25	0.001474

$$\text{Hence } K \text{ for } \tfrac{1}{3}\text{-normal} = \frac{0.001597 + 0.001474}{2} = 0.001535.$$

By the same method was the following number obtained for $\frac{1}{10}$-normal:

No. of Experiment.	Quantity of Salt taken.	Quantity of Water in cc.	Time in Minutes.	Percentage decomposed.	*K*.
1	0.1325 gram	9.03	105	8.5	...
2	"	"	120	9.5	0.00084
3	"	10.03	120	9.	...
4	"		135	10.	0.00078

$$\text{Hence } K \text{ for } \tfrac{1}{10}\text{-normal} = \frac{0.00084 + 0.00078}{2} = 0.00081.$$

A comparison of these coefficients of velocity among themselves, and with the coefficients of affinity of monochloracetic acid at the same states of dilution, reveals in an interesting way the dependence of the velocity of this decomposition at these several states of dilution upon the relative strengths of the acids concerned in this reaction.

According to Ostwald, the several coefficients of affinity of monochloracetic acid, in terms of that of hydrochloric acid as unity, are for these dilutions as follows:

Coefficients of the Affinity of Monochloracetic Acid: HCl = 1.	Coefficients of the Velocity of the Decomposition of Potassium Chloracetate.
Normal = 0.51	Normal = 0.0035
$\frac{1}{3}$-Normal = 0.07	$\frac{1}{3}$-Normal = 0.0015
$\frac{1}{10}$-Normal = 0.213	$\frac{1}{10}$-Normal = 0.0008

For the sake of the comparison the coefficients of velocity have been placed by their side. It will be seen from these numbers that the affinity of monochloracetic acid in $\frac{1}{10}$-normal dilution is about *four* times as great as it is at normal. The coefficients of velocity in the other column show just as clearly that the rate of the decomposition at $\frac{1}{10}$-normal dilution is about *one-fourth* as great as it is at normal. In other words, the velocities of the decomposition of potassium chloracetate at normal and $\frac{1}{10}$-normal states of dilution are inversely proportional to the coefficients of affinity of monochloracetic acid at the same states of dilution.

$\frac{1}{10}$-N.	N.	N.	$\frac{1}{10}$-N.
0.213	0.051	0.0035	0.0008

The equality of the two ratios is as close as could be expected, considering the fact that the two sets of numbers were arrived at by the study of altogether different chemical processes.

In the same way we have:

$\frac{1}{2}$-N.	N.	N.	$\frac{1}{2}$-N.
0.07	0.051	0.0035	0.0015

Here the inverse proportionality does not hold as strictly as in the above. This may possibly be accounted for in this way: Ostwald's numbers for normal and $\frac{1}{2}$-normal, 0.051 and 0.07 respectively, were arrived at by different methods, and hence, as he himself says, are not strictly comparable. The numbers for normal and $\frac{1}{10}$-normal, *viz.*, 0.51 and 0.213, were, however, obtained by the same method, and here we find the agreement as close as could be desired.

The Effect of Glycollic Acid upon the Rate of the Decomposition.

In studying the decomposition of the silver salt of chloracetic acid it was found that the glycollic acid exerted a slight retarding influence upon the course of the reaction. Strange to say, however, the reverse of this has been found to be true in the case of the potassium salt. That is, the effect of the glycollic acid upon the decomposition is one of acceleration rather than retardation. At present this is very nearly all that may be said concerning the influence of this substance, for the reason that, while a great deal of time and attention has been given to this particular part of the subject, the results obtained as yet are by no means as satisfactory as could be desired. This is largely owing to the fact that the

influence exerted by this compound is at best but slight; so slight, indeed, in some cases that, unless the decomposition be extraordinarily prolonged—which in some instances is impracticable—the acceleration is not accurately measurable by the means employed.

The following are some of the results obtained:

TABLE V.—*Showing the Effect of Glycollic Acid upon the Decomposition of Potassium Chloracetate.*

Temperature, 100° C.

No. of Experiment.	Potassium Salt taken.	Quantity of Water in cc.	Time in Hours.	Percentage Decomposed: Alone.	With .076 gram Glycollic Acid.
1	0.1325 gram	1	1	16 : 16.5	17.5 : 18
2	"	1	2	28 : 28.5	31 : 32
3	"	1	3	40.5 : 41	44 : 46.5
4	"	3	1	9 : ...	10 : ...
5	"	3	2	16 : ...	19 : ...
6	"	3	3	24.5 : ...	30 : ...
7	"	10	1	5 : ...	7 : ...
8	"	10	2	9 : ...	11 : ...
9	"	10	3	13.5 : ...	15 : ...

From these results it would appear that the slight acceleration produced by this substance is independent of the state of dilution. As illustrative of this compare the results of Exp. No. 1 with Nos. 5 and 9; also No. 2 with No. 6 and No. 4 with No. 8, in the above table. It would also appear from the results given in Table VI (below) that the acceleration is directly proportional to the quantity of glycollic acid present.

TABLE VI.

Time, 3 hours; temperature, 100° C.; quantity of salt in each case, 0.1325 gram: quantity of water, 2.28 cc.

	I.	II.	III.	IV.
Quant. of glycollic acid	none	0.019 gr.	0.038 gr.	0.076 gr.
Perc. of decomposition	28.	29.25	30.5	33.25
Acceleration	...	1.25	2.5	5.25

In this connection it occurred to us to try the action of other acids upon the course of the decomposition. The effect of the

following has been tried with rather remarkable results: nitric, hydrochloric, sulphuric, formic, acetic, propionic, and malonic. It has been found that those weaker than monochloracetic *accelerate* the decomposition in the order of their strength—that is, formic accelerates it more than glycollic, and glycollic more than acetic, etc.; whereas, with those stronger than monochloracetic exactly the reverse seems to be true—that is, hydrochloric and nitric *retard* the decomposition very greatly and nearly to the same extent, and both more than sulphuric, etc.

The data now at hand are not sufficient to warrant any further statement as to the influence of acids in general upon the decomposition now being studied; much less do they permit of any speculation as to the bearing of all this upon the original problem. It is believed, however, that further study in this direction will yield results of considerable value and furnish one of the most interesting chapters in the complete solution of the problem now under investigation.

TABLE VII.—*Showing the Effect of Potassium Chloride upon the Decomposition of Potassium Chloracetate.*

Temperature, 100° C.; quantity of salt in each case, 0.1325 gram.

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.
Quant. water in cc.	1	1	1	3	3	3	10	10
Time in hours	1	2	3	1	2	3	2	2
Perc. dec. (alone)	16.5	28	41	9	16	24.5	12	12
Perc. dec. with 0.0745 gram KCl	18	31	44.	9.5	16.5	28	13	12.5

The influence of potassium chloride has also been studied. About all that can be said at present is that it, like the glycollic acid, exerts a slight accelerating influence upon the decomposition, as may be seen from the results given in Table VII. It may be said in this connection that sodium bromacetate, by reason of its perfect purity and more ready decomposibility, has furnished a most excellent material with which to study the influence of both products of this reaction upon the decomposition. We hope in the near future to reach results better and more concordant than it has been found possible to obtain in this connection with potassium chloracetate.

Monobromacetic Acid.

This investigation, originally undertaken with the view to determine the relative velocities of decomposition of the silver salts of the several principal members of the series of haloid fatty acids, has so outgrown its original bounds, and so many points of interest have revealed themselves in connection with the best known of these acids—monochloracetic—as the work progressed, that while the original aim of the study has not been lost sight of, up to the present only one other acid, monobromacetic, has received attention.

Anhydrous Sodium Bromacetate.—This salt was prepared by slowly adding to a saturated aqueous solution of sodium carbonate the required quantity of the pure monobromacetic acid. The mixture was constantly stirred and its reaction tested from time to time. To the neutral solution thus obtained there was added, with constant agitation, a considerable quantity of absolute alcohol. Under these conditions the salt was obtained in the form of white, pearly leaflets of great purity. It may be dried in the usual manner upon filter-paper and finally under the desiccator. In appearance it strikingly resembles the salts of the corresponding chlor-acid. It is stable in dry air and freely soluble in cold water. Solutions of it were found to be perfectly neutral and to give no turbidity with silver nitrate. On analysis the following number was obtained for the sodium:

	Found.	Calculated for the Anhydrous Salt.
Na	14.43	14.32 per cent.

Decomposition of Sodium Bromacetate in Water.

In studying the decomposition of this salt a remarkably interesting observation was made. It was observed that when this salt was heated with small quantities of water a small quantity of a white, waxy substance resulted, which dissolved but slowly when treated with larger quantities of water, giving rise gradually, however, to an acid solution.

In order to determine the nature of this substance, about 8 grams of the sodium salt were heated for an hour and a half with about 5 cc. of water. The resulting mass was then treated with a considerable quantity of cold water to remove the sodium bromide and any other soluble products of the reaction; it was then filtered

and the white mass washed repeatedly with small amounts of cold water, dried and weighed. It was found to weigh 0.8 gram. The substance thus obtained was found to melt at 160° C.; when boiled with water it slowly dissolved; the solution became acid in reaction; and on cooling the substance partially separated out again in the form of a white powder, showing a great tendency to remain suspended in the liquid. It was found to dissolve very freely in strong ammonia, giving rise to an exceedingly soluble product.

It also dissolves in solutions of caustic potash and soda, and in order to determine its composition the larger portion of it was boiled for some time with water and the required amount of lime. The solution thus obtained was evaporated to small bulk, and while hot, filtered and set aside. After standing over night the calcium salt crystallized out in the form of white, satiny, needle-shaped crystals. These were filtered off, air-dried, and the calcium determined as the sulphate, with the following result:

	Found.	Calculated for $(CH_2OH.COO)_2Ca + 3H_2O$.
Ca	16.39	16.39 per cent.

This analysis and the mode of formation both indicate that the substance is glycollide. The fact that such a substance may be produced in such a decomposition, even in the presence of much more than the quantity of water necessary to form glycollic acid at once, is one of particular interest in this connection. It would seem to indicate that, in all such decompositions, the action of water, aside from promoting dissociation, is purely one of a secondary kind, and that, strictly speaking, such reactions are to be interpreted as taking place in two stages instead of one, as commonly represented, thus:

$$(1)\quad CH_2Br.COONa = NaBr + \underline{CH_2COO.}$$

$$(2)\quad \underline{CH_2COO} + H_2O = CH_2OH.COOH.$$

The decomposition of sodium bromacetate in water is an exceedingly rapid one. The effect of dilution upon the velocity of the decomposition is much the same as that already described at length for the salts of the chlor-acid. In the following table, No. VIII, are given the results thus far obtained. The several states of dilution at which these experiments have been tried were chosen with the view to determine the coefficients of velocity for normal, $\frac{1}{3}$-normal and $\frac{1}{10}$-normal solutions.

TABLE VIII.—*Showing the Effect of Water upon the Decomposition of Sodium Bromacetate.*

Temperature, 100° C.; quantity of salt in each case, 0.1610 gram.

No. of Exp.	Quan. of Water in cc.	Time in minutes.	Percentage decomposed.	*K.*	A.
1	0.5	8	51 : 52	.0916	$\frac{.0916 + .0513}{2} = .0714$ N.
2	1.	12	46 : 46	.0513	
3	1.5	20	54 : 54	.0388	$\frac{.0388 + .0269}{2} = .0328$ $\frac{1}{3}$-N.
4	3.	25	49 : 49	.0269	
5	5.	30	48 : 47.5	.0218	$\frac{.0218 + .0173}{2} = .0195$ $\frac{1}{10}$-N.
6	5.	300	99	...	
7	10.	40	50	.0173	

In column A of the table are given those mean values which represent the coefficients of velocity at normal, $\frac{1}{3}$-normal, and $\frac{1}{10}$-normal states of dilution. It is interesting to note in this connection that these several values stand in very much the same relation to each other that do those obtained for potassium chloracetate (see p. 481). Each of these values, however, is about twenty times greater than those obtained in the case of the potassium salt of the chlor-acid.

Thus we have:

Coefficients of Velocity for Potassium Chloracetate × 20.	Coefficients of Velocity for Sodium Bromacetate.
Normal = .0035 × 20 = .0700	.0714
$\frac{1}{3}$-Normal = .0015 × 20 = .0300	.0328
$\frac{1}{10}$-Normal = .0008 × 20 = .0160	.0195

thereby showing that, while the salts of bromacetic acid are twenty times more unstable than those of chloracetic acid, their decomposition is nevertheless retarded by water by amounts proportionate to those observed for the decomposition of the salts of the chlor-acid.

It might be well in passing to call especial attention to experiments Nos. 5 and 6, Table VIII, as showing, first, that the decomposition can reach completion and, secondly (compare time and amounts decomposed in 5 and 6), that the time required for completion is ten times as long as that required for the decomposition of one-half of the substance.

For the reason that such large amounts of decomposition could be obtained with the sodium bromacetate in such comparatively

short intervals of time, it afforded an excellent material with which to determine the influence of glycollic acid upon the velocity of the reaction.

The following results have been obtained:

TABLE IX.—*Showing the Effect of Glycollic Acid upon the Decomposition of Sodium Bromacetate.*

Temperature, 100° C.; quantity of salt in each case, 0.1610 gram; quantity of glycollic acid, 0.076 gram.

No. of Exp.	Quantity of Water in cc.	Time in Minutes.	Percentage decomposed.	Percentage taken from Table VIII.	Acceleration.	
1	0.5	8	55.	51.5	3.5	
2	1.	12	54.	46.	8.	
3	1.5	20	61.	54.	7.	
4	3.	25	55.	49.	6.	Mean = 5.2
5	5.	30	51.5	47.5	4.	
6	10.	40	53.	50.	3.	
7	50.	60	60.	55.	5.	

These results strengthen the conclusion already reached concerning the influence of this acid upon the decomposition (see p. 482). The acid slowly accelerates the decomposition, and seems in its action to be independent of the dilution. It is true that the numbers here obtained for the several dilutions do not agree among themselves quite as well as might be expected. In only one case, however, is the departure from the mean value (5.2) greater than 2; there are just the same number of departures on one side as on the other, and it should also be borne in mind that in each case it was found necessary to weigh the acid, which, it will be remembered, is deliquescent and rather difficult to preserve of uniform composition. The fact that the acceleration here produced by the glycollic acid is so nearly the same for the different dilutions, and so nearly the same also for that observed in the case of the potassium chloracetate (compare Exp. 1 with those in Table VI), renders it unnecessary to apply any correction to the coefficients of velocity given above, for the reason that this correction would apply equally to each at the several dilutions and also to both sets alike. Each would become a trifle smaller, but the same relation would hold for those of each acid among themselves.

Summary and Conclusion.

The more salient features of the decomposition of the salts of monochloracetic and monobromacetic acids, as clearly indicated by the results given in detail above, are briefly these:

1. The decomposition is greatly retarded by increasing the quantity of water, in which medium the decomposition readily takes place.

2. The decomposition is one of a simple, non-reversible kind, and may be carried to completion.

3. The decomposition, at certain states of dilution, probably at all, is independent of the nature of the base or metal in combination with the acid.

4. The coefficients of velocity of the decomposition at normal, $\frac{1}{2}$-normal and $\frac{1}{10}$-normal dilutions are inversely proportional to the coefficients of affinity of monochloracetic and monobromacetic acids at these several states of dilution.

5. The decomposition reaches a minimum at a dilution somewhat above $\frac{1}{10}$-normal (the exact point not yet determined), from which point on to a dilution of 1000 litres the decomposition is nearly constant.

6. The velocity of the decomposition is slightly accelerated by glycollic acid proportionately to the quantity of the acid present. Other acids weaker than monochloracetic have also been found to *accelerate* the decomposition in the order of their affinities. With acids stronger than monochloracetic exactly the reverse of this has been found to hold.

7. The velocity of the decomposition is also slightly accelerated by potassium chloride.

In discussing the results obtained in the case of the decomposition of silver chloracetate it was assumed by way of explanation that the salt dissociates in water, and that we have acting upon the metal, potentially at least, two acids or two acid ions, one of monochloracetic, the other of hydrochloric acid. The affinity of hydrochloric acid, in consequence of its extreme dissociation even in concentrated solution, is but slightly augmented by larger dilution, and hence in its influence upon the reaction studied may be put as a constant force tending to produce the change in question. Opposing this change, however, and therefore retarding the velocity of the decomposition, is the affinity of monochloracetic acid which, while much weaker than the hydrochloric at the beginning,

rapidly increases in strength in consequence of dilution, and consequently as the dilution increases holds the decomposition more and more completely in check.

The correctness of the above explanation has been called in question by Prof. Ostwald.[1] Unfortunately this criticism is not at hand. Through the kindness of Prof. Remsen, however, the following paragraph, which we quote below in full, and which according to the latter (Prof. Remsen) contains the essential elements of the criticism, has been brought to our notice:

"Der Verfasser diskutiert seine Versuche und gelangt zu einer etwas verwickelten Vorstellung, die hier nicht wiedergegeben werden soll. Dem Referent scheint eine andere Deutung wahrscheinlich. Die Frage, welcher von den beiden Anteilen des Salzes, der dissocierte oder der nicht dissocierte, der Zerzetzung erfährt, ist wahrscheinlich dahin zu beantworten, dass es der nicht dissocierte ist. Dieser nimmt aber mit steigender Verdünnung sehr schnell ab, und daraus würde sich die sehr starke Abnahme der Reaktionsgeschwindigkeit erklären."

After a very careful study of the above we are inclined to doubt if, after all, there is any essential difference between the explanation originally offered, it may be in rather complicated form, by one of us, and that so tersely expressed by Prof. Ostwald in the above paragraph.

From our standpoint it would seem to be an explanation of an explanation, if we may use this expression, rather than a totally different solution of the problem. If we understand him rightly, his view of the decomposition as applied to the case of potassium chloracetate would be somewhat as follows: When potassium chloracetate is brought into solution in water, dissociation takes place, and there exist in the solution at the same time and side by side ions of the dissociated portion of the salt (*viz.*, the potassium and the chloracet ion), together with a certain quantity of the undissociated portion of the salt composed of molecules of potassium chloracetate as such. Of these two portions it would seem that the latter only undergoes decomposition; it, however, rapidly decreases on dilution, and hence the marked falling off in the velocity of the reaction. If we accept this explanation, according to which the velocity of the reaction would be conditioned proportionately to the mass of undissociated potassium chloracetate

[1] Ztschr. phys. Chem. **11**, 431.

present, then the question at once suggests itself, what will determine the quantity of undissociated salt at any given state of dilution? Now, plainly the amount of undissociated salt at any given dilution depends upon the quantity which is *dissociated*, and this latter will be directly proportional to the affinity of the chloracetic acid at that state of dilution, for the reason that the affinity of this and other acids depends upon the extent to which it and their salts are dissociable. It is just in this particular, therefore, that Prof. Ostwald's explanation, if carried to what would seem its rightful limit, presents many points of agreement with that put forward by one of us.

It would seem upon either hand ultimately to resolve itself into a question of relative affinities, and while it is to be admitted that Prof. Ostwald's conception of the decomposition, or at least the statement of it, has much in its favor so far as clearness is concerned, it is to be doubted if anything has really been gained, beyond a mere succinct expression, over the hypothesis as originally put forward. The true cause of the decomposition, whether it be of the whole or only a portion of the salt, is to be sought in the fact that the acid ion of hydrochloric acid, Cl, has under certain conditions sufficient strength to break down the molecule. The extent to which, however, this decomposition can take place depends very intimately upon the quantity of water present, for the reason that, according to Ostwald, the extent of the dilution determines the quantity of dissociated and non-decomposable salt, and therefore also the quantity of that which is undissociated and decomposable. But the extent of this dissociation is also a measure of the relative affinity of monochloracetic acid, and hence it is that the several velocities of the decomposition at different dilutions should be inversely proportional to the several coefficients of the affinity of this acid at corresponding dilutions.

In both explanations the dissociation of salts in aqueous solution is the central idea: the one stops at this point, the other goes on and attempts to establish connection between the decomposition under consideration and certain fundamental properties of a certain class of chemical compounds—properties which have already been proven to be dependent upon the amount of dissociation, *i. e.*, the affinity of acids.

If it were not known that the weaker acids increase in strength upon dilution, and, further, if this increase in strength were not

known to be due to the further dissociation produced in the compound by water, then indeed Prof. Ostwald's explanation might be said to be absolutely essential to a thorough understanding of this reaction. As it is, however, the cause producing the change in the affinity of any given acid at various dilutions has been accepted as a well established principle, and the endeavor has been made to connect with the effects of this cause such changes in the velocity of the decomposition as have been observed.

It should be said in this connection that the hypothesis as originally put forward had for its object solely to account for the retarding influence of water upon the velocity of the decomposition. It was not claimed for it that it would in any way account for the absolute amount of decomposition in any case, or offer any explanation for any constant difference which might be found to exist between the rates of decomposition of the salts of different acids—such, for example, as the marked and almost constant difference in the velocity of decomposition already shown to exist between the salts of monobrom- and monochloracetic acids. On the other hand it is doubtful if any hypothesis, in the light of our present knowledge, can be advanced which will rightly embrace these two markedly different aspects of the phenomena here presented. The three acids, hydrochloric, hydrobromic, and hydriodic, have practically the same affinity and yet they differ very widely in the order of stability. Now so far as we are aware no theory seems capable of grasping at once these two sets of facts. About all that can be done at present is to hold up these two properties, activity and stability, as two entirely distinct and different properties of compounds. Monochloracetic acid and monobromacetic acid furnish us with a parallel case; the salts of the latter decompose about twenty times as rapidly as do those of the former, and yet the coefficients of velocity of each exhibit very nearly the same relation among themselves at corresponding states of dilution.

The hypothesis under discussion was therefore, as stated above, originally advanced solely to explain the relation found to exist between the several coefficients of the velocity of the decomposition of the salts of any given acid at various states of dilution. This it has been found to do within certain limits. As may be seen on p. 486, it has been found that, for the salts of monobrom- and monochloracetic acids, at normal, $\frac{1}{2}$-normal, and $\frac{1}{10}$-normal

states of dilution, the coefficients of the velocity of decomposition for each vary inversely as the coefficients of affinity of each of these acids at the several dilutions above named. It can scarcely be that the remarkable coincidence with which we are here dealing is simply a matter of chance or the result of accident. It may be argued against this hypothesis that the retarding effect produced by water stops far short of the point at which it, the water, exerts its greatest influence, by virtue of its dissociating power, upon the strength of monochlor- aud monobromacetic acids. For example, Ostwald has determined the affinity of these acids by means of their electrical conductivity, at the following dilutions, with these results:

	4 litres.	32 litres.	256 litres.
Monochloracetic acid	6.98	17.3	37.8
Monobromacetic acid	...	16.1	36.8

From this we see that, at a dilution of 256 litres, each of these acids is between five and six times as strong as it is at a dilution of 4 litres, and doubtless fully seven times as strong as it is at a dilution of 3 litres. Hence in the light of our hypothesis we ought to expect the coefficient of velocity for the decomposition of potassium chloracetate at the higher dilutions at which it has been studied, to sink as low as .0002 or .00025, which is about one-seventh of .0015—the coefficient of velocity for $\frac{1}{3}$-normal. As a matter of fact, however, no coefficient as small as either of the above has been obtained. As above stated, the minimum of decomposition seems to be reached at some dilution not very far removed from that of $\frac{1}{10}$-normal, at which the coefficient of velocity has been found to be .0008. From this dilution on the velocity of decomposition remains nearly constant.

It is to be remembered in this connection, however, that the coefficients of affinity of these acids for these higher states of dilution have been determined by the electro-chemical method, which in point of delicacy and nicety of measurement and in freedom from the disturbing influences of secondary reactions far exceeds that of any other chemical process; and it is much to be doubted if any strictly chemical process, such as the one now under investigation, will ever be found to yield such results, especially at these higher dilutions, as the constants obtained by purely physical methods would seem to require. This certainly has been found to apply to the determination of the affinity of acids. When

determined by strictly chemical methods, the affinities of strong acids as a rule appear too strong, and weak acids too weak, in consequence of the presence of neutral salts and the influence of secondary reactions.

As we have already seen, the reaction under consideration is by no means free from the disturbing influence of the products of the decomposition; and while the results thus far obtained would seem to indicate that this disturbance is the same for different dilutions and therefore to be ignored, such may not indeed upon further investigation prove to be the case. On the other hand, it is easily conceivable that disturbing influences, each so small as to be imperceptible by the methods employed, might, by their combined action, altogether vitiate the final result.

In conclusion, it may be said that the present investigation was not undertaken with the view to establish or uphold any particular hypothesis, but simply to arrive, if possible, at the general law, if such there be, underlying such decompositions as have formed the subject of the present study.

The experimental difficulties in the way of arriving at results altogether satisfactory, while by no means insurmountable, are nevertheless such as to render progress along this line exceedingly slow. It has been found necessary, therefore, to modify the conditions and methods of experiment from time to time during the progress of the investigation. In the same way, as the investigation proceeds, it may also prove necessary to modify, or perhaps completely to change, the hypothesis originally advanced by way of explanation of the results first obtained. In the event that such indeed prove to be the case, we shall be found quite as willing to change our theoretical notions as we have been to modify for the better our methods of experimentation. Neither is to be regarded in any sense as final, but each rather as a means to an end.

A. AND M. COLLEGE OF KENTUCKY, LEXINGTON, *May*, 1893.

ON THE DOUBLE CHLORIDES OF LEAD AND AMMONIUM.

By Wyatt W. Randall.

In a paper published in 1889,[1] in which he calls attention to a law apparently holding good for all the so-called double halides of the alkali metals, Remsen says[2]: " . . . The examples given in the above tables are fair representatives of the whole class of double halides, except that all ammonium salts are omitted. The double halides formed with halogen salts of ammonium are very numerous, and many of them are quite complicated. There are, indeed, ammonium salts known, belonging to all the classes above mentioned, but there are many others which do not belong to these classes. Considering the power of ammonium salts in general to form complex compounds with metallic salts, one cannot avoid the conclusion that there is some power in nitrogen which makes the formation of these compounds possible. The position of the double halides containing ammonium is certainly exceptional."

His attention was, therefore, for the time being withdrawn, so far as actual investigation was concerned, from those compounds which contain the haloid salts of ammonium. In his second paper on this subject[3] he again declines strictly to consider these salts either as exceptions to or as confirmations of the law of composition, preferring to postpone the question of their nature and constitution until a more convenient season. At the suggestion of Professor Remsen I have made a beginning in the work of reinvestigating the composition of the double halides containing ammonium, by examining the case of the salts formed by the union of lead and ammonium chlorides. An additional interest is given to the investigation of these compounds by reason of the fact that, after most careful work, there was in no case obtained by Herty[4] or by Wells[5] a double halide of lead and potassium (or sodium) which did not accord with the law of composition stated by Remsen.

The only double chlorides containing lead and ammonium which I have been able to find described in the literature of the

[1] This Journal **11**, 291. [2] *Ibid.* **11**, 296. [3] *Ibid.* **14**, 81.
[4] *Ibid.* **14**, 126. [5] Am. J. Sci. **45**, 121.

subject are enumerated in a series of papers[1] by G. André, by whom they were prepared. The formulæ of these salts are, according to this author, (*A*) $PbCl_2.9NH_4Cl.1\frac{1}{2}H_2O$ ("2PbCl,9AzH$_4$Cl,3HO"); (*B*) $2PbCl_2.11NH_4Cl.3\frac{1}{2}H_2O$ ("4PbCl,11AzH$_4$Cl,7HO"); (*C*) $2PbCl_2.9NH_4Cl.2\frac{1}{2}H_2O$ ("4PbCl,9AzH$_4$Cl,5HO"); (*D*) $PbCl_2.6NH_4Cl.H_2O$ ("PbCl,3AzH$_4$Cl,HO"); (*E*) $PbCl_2.18NH_4Cl.4H_2O$ ("PbCl,9AzH$_4$Cl,4HO"); (*F*) $PbCl_2.10NH_4Cl.H_2O$ ("PbCl,5AzH$_4$Cl,HO").

There is no one of these which is not an exception to Remsen's law of composition. One is naturally struck by the fact that no salt of comparatively simple formula is to be found in the list; nevertheless, double halides of complex character are by no means unknown, and the substitution of ammonium for sodium, potassium, etc., introduces a new factor likely to do away with the value of *a priori* reasoning.

Efforts have therefore been made to prepare the various double chlorides of lead and ammonium described by André, and, it may as well be said here, without success. The only definite double salts obtained will be shown to have been in accordance, as regards composition, with Remsen's law. I have called the salts described by André by the letters *A*, *B*, *C*, etc.

M. André seems to have relied entirely upon the results of his analyses to establish the definiteness of the salts which he prepared. There does not appear to have been any microscopic examination made; in fact hardly any reference to crystalline form, even, is to be noticed in his papers. Each of the salts described in this paper was subjected to crystallographic examination by Mr. E. B. Mathews, of the Geological Department of this University.[2] The results of these examinations will be given in their proper places.

Salt A.

Lead chloride dissolves more readily in a solution of ammonium chloride than in pure water, and with greater ease the more concentrated the solution. If now a solution of sal ammoniac which has been saturated in the cold be heated to boiling and saturated with lead chloride, there separates, on cooling, a salt which,

[1] Compt. rend. **96** (1883), 435; Bull. Soc. Chim. (Paris) **40** (1883), 14; Ann. chim. phys. [6] **3**, 104.

[2] It is but a small return to Mr. Mathews for his kindness in this matter to acknowledge here my appreciation of his services.—W. W. R.

according to André, has the composition represented by the formula $PbCl_2.9NH_4Cl.1\frac{1}{2}H_2O$. To test the accuracy of this formula, efforts were made to prepare the salt by crystallization from solutions containing different amounts of lead chloride. A solution of sal ammoniac was made, saturated in the cold. Successive portions of this, of 250 cc. each, were taken and, after being raised to the boiling-temperature, were treated with definite quantities of crystallized chloride of lead. To the first portion were added 2 grams of lead chloride; to the second, 3; to the third, 4; to the fourth, 5; to the fifth, 10; to the sixth, 15 grams. A clear solution was obtained practically at once in the first four cases; in the fifth, the deposit disappeared after a few moments; in the last case, continued heating for a half-hour failed to produce a clear solution. An additional 50 cc. of the sal-ammoniac solution was therefore added and the heating continued. Ultimately the solution became perfectly clear. It would thus appear that the limit of solubility is reached when 15 grams of lead chloride are dissolved in 300 cc. (or 12.5 grams in 250 cc., to bring in accord with the other ratios). Each of these solutions was then set aside to cool, the beakers in which they were contained being placed within other beakers and surrounded with cotton-waste in order that the cooling might take place slowly and evenly. A deposit of crystals was formed in each case; these deposits I have numbered from **1** to **6**, beginning with that deposited from the solution which contained but 2 grams of lead chloride, and ending with that which was saturated.

The salts **1–5** were deposited in fine, highly refracting, white crystals, and looked very much alike; it will be shown that they possessed the same composition. Salt **6** crystallized out in much larger, more or less clear, orthorhombic pyramids. Under the microscope it was seen that **5** differed from **6** in having a larger development of the brachypinacoid and in having a basal plane. The $\frac{1}{2}P\infty$ faces, too, occur in different positions in the two salts.

Determinations of lead were made as follows: A weighed quantity of the salt was treated in a porcelain crucible with a few drops of pure concentrated sulphuric acid. Effervescence of hydrochloric acid having ceased, the crucible was placed on a triangle within an iron crucible and the temperature gradually raised until the ammonium sulphate had been volatilized. It was then carefully heated over the free flame for a few moments, allowed to

cool, again treated with acid and again heated in the iron crucible. The crucible and its contents were allowed to cool in a desiccator and were then weighed. In several instances another heating with acid and a second weighing were resorted to, but in no case was there a noteworthy difference in the two weights. In two instances the heating over the free flame was allowed to proceed until the crucibles were red-hot. There resulted a considerable dissociation of the lead sulphate and absorption of the lead by the glaze of the crucible. Further heating with sulphuric acid failed to remove the stain in the glaze, and so the results of these determinations were discarded.

The determinations of ammonia were made by decomposing a weighed quantity of the salt with an alkali and absorbing the ammonia evolved in a standard solution of hydrochloric acid. In the case of the first determination of ammonia in salt **6**, the salt was merely boiled with a strong solution of sodium hydroxide; in the case of the second, steam was conducted through the boiling alkali solution; afterwards milk of lime was used instead of caustic soda, and a current of steam steadily passed through the boiling liquid. These variations in the method employed may serve in part to explain the lack of perfectly satisfactory agreement among these three determinations. All the other determinations of ammonia were made with milk of lime as reagent.

The figures which follow are those obtained on analysis of salts **1–6**.

Salt **1**.

I. 0.29156 gram salt gave 0.28852 gram $PbSO_4$.
II. 0.5205 gram salt gave 0.5144 gram $PbSO_4$.

Salt **2**.

I. 0.31619 gram salt gave 0.31201 gram $PbSO_4$.
II. 0.41117 gram salt gave 0.40607 gram $PbSO_4$.

Salt **3**.

I. 0.5555 gram salt gave 0.54671 gram $PbSO_4$.
II. 0.52587 gram salt gave 0.51888 gram $PbSO_4$.

Salt **4**.

I. 0.37918 gram salt gave 0.37411 gram $PbSO_4$.
II. 0.52197 gram salt gave 0.51435 gram $PbSO_4$.

Salt **5.**

I. 0.91077 gram salt gave 0.89967 gram $PbSO_4$.
II. 0.60435 gram salt gave 0.59779 gram $PbSO_4$.
III. 0.80643 gram salt gave 0.79875 gram $PbSO_4$.
IV. 0.80187 gram salt gave 0.02483 gram NH_3.
V. 0.5969 gram salt gave 0.01875 gram NH_3.

Salt **6.**

I. 0.43165 gram salt gave 0.34003 gram $PbSO_4$.
II. 0.55922 gram salt gave 0.43943 gram $PbSO_4$.
III. 0.41978 gram salt gave 0.3326 gram $PbSO_4$.
IV. 0.67897 gram salt gave 0.05907 gram NH_3.
V. 0.41747 gram salt gave 0.03733 gram NH_3.
VI. 0.6905 gram salt gave 0.05957 gram NH_3.

Percentages.

	Salt **1.**	**2.**	**3.**	**4.**	**5.**	**6.**
Pb.—I.	67.58	67.38	67.21	67.38	67.45	53.79
II.	67.49	67.44	67.38	67.29	67.55	53.66
III.	...	...	...	...	67.64	54.10
NH_3.—I.	...	...	...	...	3.09	8.70
II.	...	...	...	...	3.14	8.94
III.	...	...	...	...	...	8.63

	Calculated for $PbCl_2.9NH_4Cl.1\frac{1}{2}H_2O$.	$(PbCl_2)_2.NH_4Cl$.	$PbCl_2.2NH_4Cl$.
Pb	26.32	67.91	53.76
NH_3	19.45	2.79	8.85

The chief conclusions to be drawn from these results are: (1) that the salts **1**, **2**, **3**, **4** and **5** are identical and consist almost entirely of the compound $(PbCl_2)_2.NH_4Cl$, but having mixed in with this compound a trace of ammonium chloride which tends to lower the percentage of lead and to raise that of ammonia; (2) that salt **6** consists practically entirely of the compound $PbCl_2.2NH_4Cl$. Under the microscope the salts appear homogeneous. On heating salts **5** and **6** in dry test-tubes, there was scarcely a trace of water given off in the case of the latter and none whatever in the case of the former, although sublimation was very marked.

The conclusion seems, therefore, certain that no salt at all corresponding to that described by André and distinguished by the title *A*, was produced, and, as the conditions were varied to a

great degree in order that the proportion between the quantities of the two chlorides should give an opportunity for the production of a variety of salts, it seems evident that the salt *A* as prepared by André was a mixture and not a chemical unit.

Salts B, C, and D.

By saturating with lead chloride a solution of ammonium chloride which had been saturated at the boiling-point, and then allowing it to cool, André claims to have prepared three different double salts by varying slightly the conditions of the experiment. These salts I have designated by the letters *B*, *C*, and *D*, and their formulæ are, respectively, $2PbCl_2.11NH_4Cl.3\frac{1}{2}H_2O$; $2PbCl_2.9NH_4Cl.2\frac{1}{2}H_2O$; and $PbCl_2.6NH_4Cl.H_2O$. To be a little more accurate, salts *B* and *C* were obtained under identical conditions; while salt *D* consisted of those crystals which were first deposited, care being taken to remove them at once from the solution. The directions given for the preparation of these salts involve the solution of "about" 90 grams of lead chloride in a solution of "about" 200 grams of sal ammoniac in "about" 200 cc. of water. Water, even at the boiling-point, will not dissolve its weight of ammonium chloride, much less will this amount of the two chlorides be taken up at the same time. The directions cannot therefore be followed exactly. The method employed was the following:

100 cc. water was saturated at the boiling-point with ammonium chloride; lead chloride was then added until the solution would no longer dissolve it. A very little hot saturated sal-ammoniac solution was then added to dissolve the slight residue of lead chloride, after which the liquid was heated until perfectly clear. The beaker was so arranged that the cooling took place very gradually. As soon as the deposition of crystals had fairly begun an attempt was made to decant the mother-liquor, the result being the mass almost solidified, and new layers of crystals settled upon those first deposited. This upper mass was carefully removed and the salt first deposited was separated as far as possible, was dried and examined. Under the microscope it was seen to be a mixture of an isotropic with an orthorhombic salt. The isotropic salt corresponded in its crystalline form with ammonium chloride, while a determination of the axial ratio of the orthorhombic salt showed it to be lead chloride. The former salt

was present in excess. An analysis of this salt ("**7**") gave the following figures:

I. 0.2844 gram salt gave 0.14353 gram $PbSO_4$.
II. 0.35156 gram salt gave 0.18295 gram $PbSO_4$.
III. 0.30707 gram salt gave 0.15558 gram $PbSO_4$.

The percentages of lead are therefore 34.46, 35.54, and 34.6, respectively. André's salt, $2PbCl_2.11NH_4Cl.3\frac{1}{2}H_2O$, should contain 34.28 per cent. of lead. It therefore seems extremely probable that the salt obtained by André under these conditions was also a mixture, and not a definite compound.

In order to test this view more thoroughly, two more attempts were made to prepare a definite salt. Instead of decanting the solution, however, a circular piece of filter-paper cut so as to fit the beaker closely, was slipped into the solution as soon as the crystallization had proceeded far enough, and was allowed to sink down and rest upon the crystalline deposit. A perforated porcelain filtering-plate, previously warmed, was then lowered upon the paper to keep it in place and the crystallization allowed to proceed. After the liquid was cold the crystals which had been caught under the paper were separated and dried. They were marked "**8**" and "**9**," respectively. Both were found to be mixtures on examination under the microscope, and on analysis gave the following figures:

I. 0.31627 gram of salt **8** gave 0.18545 gram $PbSO_4$.
II. 0.50981 gram of salt **8** gave 0.29922 gram $PbSO_4$.
I. 0.31288 gram of salt **9** gave 0.2068 gram $PbSO_4$.

The percentages of lead obtained were therefore: for salt **8**, 40.04 and 40.08, and for salt **9**, 45.13.

The only conclusion to be drawn from these results is, that if one were to perform the experiment a sufficient number of times, salts of almost any desired composition could be obtained. Analysis alone is incapable of determining with certainty the question of such a salt's individuality.

All the efforts made to prepare from lead chloride definite double chlorides of lead and ammonium of the kind described by André, by following his directions—or such modifications of these as seemed necessary or advisable—were therefore unsuccessful. There are, however, other salts described by him as formed in a solution of ammonium chloride which has been treated with litharge and heated. These salts will be here designated as *E*

and *F*, their composition being thus represented by André: *E*, $PbCl_2.18NH_4Cl.4H_2O$; and *F*, $PbCl_2.10NH_4Cl.H_2O$. In addition to these, the salt *D*, $PbCl_2.6NH_4Cl.H_2O$, is described as a product of this action under certain conditions. To put it more definitely: if a solution of 100 grams of ammonium chloride in 200 cc. of water is heated to boiling, treated with 25 grams of litharge, boiled a few moments to dissolve the oxide, filtered, and allowed to cool, the salt *E* is said to be deposited. If the boiling is continued 1½ hours, *F* is the product; if the solution is boiled 3 hours, *D* is deposited. It will be seen that the salt with the largest proportion of ammonium chloride is that formed when the amount of lead chloride in the solution is very slight, and that as the decomposition of the litharge proceeds, salts with a larger proportion of lead chloride are produced. Judging by the results obtained when lead chloride itself was added to solutions of ammonium chloride saturated in the cold—in other words, comparatively dilute solutions—this should be the case if the deposit were a *mixture;* but *not*, if the salt formed were a definite compound. For the salt $(PbCl_2)_2.NH_4Cl$ was obtained in each experiment of the first series until the solution was saturated with lead chloride, when the salt $PbCl_2.2NH_4Cl$ was formed. The value of such an argument is, of course, not great; nevertheless, if it is found to agree with the results of experiments, it must be taken into account.

The litharge used was prepared by precipitating a solution of lead nitrate with ammonium carbonate, and then heating the well-washed lead carbonate to 300° in an air-bath. The proportions given above were used, and a series of salts was obtained which were designated by the numbers **10–14**. After the litharge had been added to the hot sal-ammoniac solution, the boiling was continued but a few minutes, in the case of salts **10** and **11**. The solution was then quickly filtered with the aid of a hot-water funnel, was again heated until the lead chloride which had begun to crystallize out had redissolved, and was then allowed to cool slowly, as in the former series of experiments, care being taken, as before, to secure the crystals first deposited. In the preparation of salts **12**, **13** and **14**, the only variation on this method was, that the boiling with the litharge was continued for 1½, 1½, and 3 hours, respectively.

On examination under the microscope it was seen that each of

these salts was a mixture. **10** and **11** were composed very largely of the regular crystals of ammonium chloride. The lead chloride present had crystallized first, and was almost entirely in the form of enclosure in the crystals of sal ammoniac. The following figures were obtained on analysis:

0.23242 gram salt **10** gave 0.15188 gram $PbSO_4$.

0.25134 gram salt **11** gave 0.1775 gram $PbSO_4$.

The percentages of lead are: for **10**, 44.62; for **11**, 48.22.

Under these conditions the salt obtained by André had a composition to be represented by the formula $PbCl_2.18NH_4Cl.4H_2O$, having only 15.76 per cent. of lead. It appears, therefore, very likely that had the growth of the ammonium-chloride crystals about the lead chloride been allowed to proceed without hindrance, similar mixtures could have been obtained in these cases. In the case of salt **12** it was seen that the two constituents were present as before, the orthorhombic being in the form of an enclosure within the regular. There was apparent also a slight difference in habit in the regular constituent. The proportion of the two when examined under the microscope was seen to be about half-and-half. No analysis of this salt was made. Salts **13** and **14** were found to be very much alike. Each appeared almost homogeneous when examined under the microscope; the regular constituent being present only in very small amount. In the finer portion of **13** the ammonium-chloride crystals seemed by actual count to make up only 2–5 per cent. of the whole. Analyses of **13** gave the following figures:

I. 0.41035 gram salt gave 0.30119 gram $PbSO_4$.

II. 0.29413 gram salt gave 0.21534 gram $PbSO_4$.

III. 0.28432 gram salt gave 0.20632 gram $PbSO_4$.

IV. 0.34766 gram salt gave 0.25145 gram $PbSO_4$.

V. 0.31104 gram salt gave 0.03267 gram NH_3.

VI. 0.24866 gram salt gave 0.03528 gram NH_3.

Percentages.

	I.	II.	III.	IV.	V.	VI.
Pb	50.12	50.00	49.55	49.39	...	...
NH_3	...	...	...	...	10.50	14.19

The fact that these analyses were made in the order given may, to some extent at least, account for the uniform decrease in the percentage of lead and rise in that of ammonia; the finer, heavier

particles of the lead salt tending, as the vessel was handled, to sink to the bottom of the weighing-glass and leave the larger, lighter ammonium chloride at the surface, whence it was poured off into the crucible, etc. The percentages agree with those required for a mixture of one part of ammonium chloride with about two parts of the salt $PbCl_2.2NH_4Cl$.

In the case of salt **14** the crystallization seemed to have progressed more slowly and evenly; the salts were quite distinct and the individual crystals were well formed. Mr. Mathews therefore undertook a mechanical separation of the two constituents under the microscope, making use of the finer portions of the mixture. The regular crystals were weeded out and were labeled "**15**," while the practically homogeneous, orthorhombic portion ("**16**") was analyzed with the following results:

I. 0.24692 gram salt gave 0.24032 gram $PbSO_4$.

II. 0.37836 gram salt gave 0.36934 gram $PbSO_4$.

	Calculated for $(PbCl_2)_2.NH_4Cl$.	Found. I.	II.
Pb	67.91	66.46	66.66

It appears, therefore, that salt **16** is practically identical with salts **1–5**, or, in other words, that the same product is obtained whether we dissolve lead chloride or lead oxide in a hot, unsaturated solution of ammonium chloride; provided that in the latter case the boiling be continued for sufficient time to decompose most of the oxide. The amount of ammonium chloride deposited with the double salt depends upon the concentration, method of crystallizing, etc.

Summary.

As a result of this work it seems justifiable to say that the salts described by André as definite compounds were, on the contrary, mixtures of various compositions. The only salts which on microscopic examination proved to be homogeneous, definite substances were shown to be in harmony, as regards composition, with Remsen's law; their formulæ being respectively $(PbCl_2)_2.NH_4Cl$ and $PbCl_2.2NH_4Cl$. These results acquire fresh interest in view of the fact that they seem to indicate that some, at least, of the double salts containing ammonium follow the same law of composition as those of the other alkalies.

It is hoped in the near future to examine the double bromides of lead and ammonium. These, like the chlorides, have been

described by André as possessing formulæ utterly at variance with Remsen's Law of Composition.

In conclusion, I should express my indebtedness to Professor Remsen for many useful suggestions given by him during the course of the work.

JOHNS HOPKINS UNIVERSITY, *May*, 1893.

Contributions from the Kent Chemical Laboratory of the University of Chicago.

DERIVATIVES OF NITROGEN HALOGEN COMPOUNDS.

SECOND PAPER.

BY FELIX LENGFELD AND JULIUS STIEGLITZ.

In a preliminary paper[1] we stated that two compounds are formed by the action of sodium methylate on succinimide bromide. Their composition seemed to indicate that the lower-melting is formed by the replacement of bromine by methoxyl and the addition of one molecule of methyl alcohol, and the other by an analogous replacement of bromine by a succinimide residue,

$$\left(-N<\begin{matrix}COCH_2\\ |\\ COCH_2\end{matrix}\right),$$

and a similar addition of methyl alcohol. Further investigation has shown that such is the case and that *a molecular rearrangement occurs in the course of the reaction.* The method of formation suggested the constitution $\begin{matrix}CH_2.CO_2CH_3\\ |\\ CH_2.CON<\begin{matrix}H\\ OCH_3\end{matrix}\end{matrix}$ for the compound melting at 33.5°. It was therefore treated both with acids and with alkalies to obtain α-methylhydroxylamine. It yielded β-amidopropionic acid, however, instead. This alone does not prove that it is not an hydroxylamine derivative. Lossen[2] and his pupils found that some hydroxamic acids readily lose carbon dioxide and give amido-compounds—dibenzhydroxamic acid, for

[1] This Journal **15**, 215.

[2] Ann. Chem. (Liebig) **175**, 313 *et seq.*

instance, giving aniline. Lassar-Cohn[1] obtained anthranilic acid by the action of alkali on phthalylhydroxylamine. Beckmann[2] has shown that similar rearrangements are effected by acids. Toward mild alkalies the substance acts like the methyl ether of a monobasic acid, but this does not prove that it is not

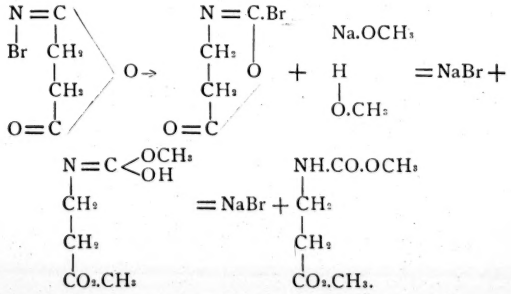
$$\begin{array}{l} CH_2.NH.CO_2CH_3 \\ | \\ CH_2.CO_2.CH_3, \end{array}$$

the carbalkoxyl group (—COOR) of the carbamic ethers being very stable.

The methyl ether of carbomethoxy-β-amidopropionic acid was therefore prepared from methyl-β-amidopropionate and methylchlorformate,

$$2NH_2.CH_2.CH_2.CO_2.CH_3 + ClCO_2.CH_3 =$$
$$CH_3.CO_2.CH_2.CH_2.NH.CO_2.CH_3 + HCl.NH_2.CH_2.CH_2.CO_2.CH_3.$$

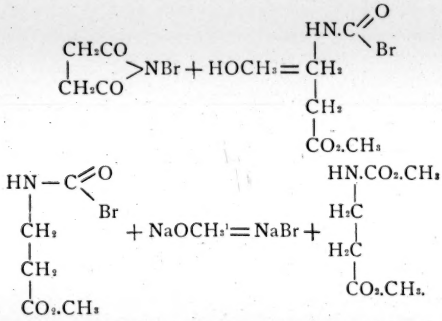
The two compounds were identical. There is, therefore, when sodium methylate acts on succinimide bromide, a rearrangement similar to that produced by the action of caustic alkalies on amide bromides, as first shown by Hofmann,[3] and more fully studied by Hoogewerf and Van Dorp.[4] The latter chemists have likewise prepared β-amidopropionic acid from succinimide by means of bromine and an alkali.[5] The formation of methyl carbomethoxy-β-amidopropionate may be represented as follows:



This scheme agrees best with the rearrangement of nitrogen compounds studied by Beckmann and others.

[1] Ann. Chem. (Liebig) **205**, 303.
[2] Ber. d. chem. Ges. **19**, 988; **20**, 1507; Ann. Chem. (Liebig) **274**, 1, etc.
[3] Ber. d. chem. Ges. **15**, 762. [4] Recueil trav. chim. **9**, 33; **10**, 4. [5] *Ibid.* **10**, 5.

It is, however, possible that the reactions are the following:

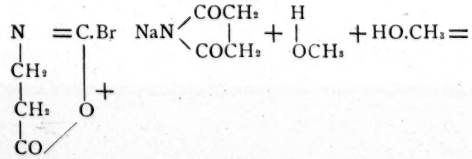


The study of methyl carbomethoxy-β-amidopropionate made it seem probable that the compound melting at 65.5° is the dimethyl ether of succinylo-β-ureidopropionic acid,

$$HO_2C.CH_2.CH_2.CONH.CONH.CH_2.CH_2.CO_2H,$$

a similar molecular rearrangement having taken place.[2] On saponification with alkalies it yields succinic and β-ureidopropionic acids. Treated with hydrochloric acid it gives a compound $C_4H_6N_2O_2$, melting at 272°.[3] The composition of this substance agreeing with that of β-lactylurea which should be readily formed from dimethyl succinylo-β-ureidopropionate, β-lactylurea was made synthetically and the two were found to be identical. There can therefore be no doubt that the substance melting at 65.5° is $CH_3.O_2C.CH_2.CH_2.CONH.CONH.CH_2.CH_2.CO_2.CH_3$. This establishes the view already expressed,[4] that sodium succinimide can play the same rôle as sodium methylate.

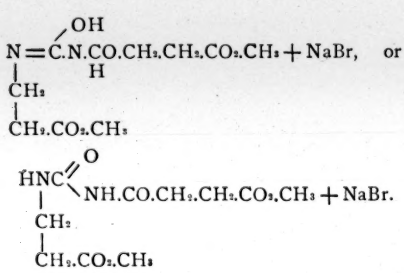
The reaction is probably as follows:



[1] The rearrangement is effected by the sodium methylate.

[2] Its formation is similar to that of acetylmethylurea observed by Hofmann in the action of sodic hydrate on acetamide bromide. Ber. d. chem. Ges. **14**, 2725.

[3] This Journal **15**, 221. [4] *Ibid.*



$$N=C(OH).N(H).CO.CH_2.CH_2.CO_2.CH_3 + NaBr, \quad \text{or}$$

$$CH_2(CH_2.CO_2.CH_3).N=C(OH).N(H).CO.CH_2.CH_2.CO_2.CH_3 + NaBr$$

$$HNC(=O)N.NH.CO.CH_2.CH_2.CO_2.CH_3 + NaBr.$$

$$CH_2(CH_2.CO_2.CH_3)$$

Experimental Part.

Methyl carbomethoxy-β-amidopropionate, $CH_3O_2C.NH.CH_2.CH_2CO_2CH_3$.—This compound has already been described by us.[1] In portions of 3 grams it was heated to 120°–130° in sealed tubes with hydrochloric acid (7–8 grams) for four hours. On opening the tubes a gas, identified as carbon dioxide, escaped in quantity. The liquid contents of the tubes were evaporated on the water-bath to a syrup which, on cooling, solidified to a crystalline mass. This was washed with chloroform, placed over sodic hydrate to remove traces of hydrochloric acid, recrystallized from boiling absolute alcohol, washed first with alcohol and then with ether, and finally brought on clay plates. Addition of ether to the alcoholic filtrate precipitated more of the same substance. Analysis and its behavior showed it to be the *hydrochloride of β-amidopropionic acid*, $HCl.NH_2.CH_2.CH_2.CO_2H$. It forms white crystals which under a microscope are seen to be irregular plates. It melts at 122.5°. Analysis gave the following figures:

0.2557 gram substance gave 0.1541 gram H_2O and 0.2671 gram CO_2.

	Calculated for $C_3H_8NO_2Cl$.	Found.
C	28.69	28.49
H	6.37	6.69

Decomposition of methyl carbomethoxy-β-amidopropionate by hydrochloric acid at ordinary pressures, or by potassic hydrate and subsequent addition of hydrochloric acid, gave the same salt together with some carbomethoxy-β-amidopropionic acid, which, being easily soluble in chloroform, can be separated from it

[1] This Journal **15**, 217.

without difficulty. The reaction is therefore not complete. In sealed tubes the yield, however, is quantitative according to the equation,

$$\begin{array}{l}\mathrm{NH.CO_2.CH_3}\\ |\\ \mathrm{CH_2.CH_2.CO_2.CH_3}\end{array} + 2H_2O + HCl = \begin{array}{l}\mathrm{NH_2.HCl}\\ |\\ \mathrm{CH_2.CH_2.COOH}\end{array} + CO_2 + 2CH_3OH.$$

The substance thus obtained is undoubtedly the hydrochloride of β-amidopropionic acid. We obtained the acid from it and made it from the acid prepared by Hoogewerf and Van Dorp's[1] method. It crystallizes easily and can be used as an aid in identifying β-amidopropionic acid.

β-Amidopropionic acid.—4 grams of the hydrochloride were dissolved in water, boiled with an excess of lead oxide,[2] evaporated to dryness on the water-bath, the powdered mass extracted with boiling water, which was cooled, filtered, and the lead precipitated by hydrogen sulphide. The residue on evaporation was dried on a clay plate. It weighed 2.5 grams and was pure β-amidopropionic acid. It melts with decomposition and evolution of gas at 206°–207°, heating rapidly. The melting-point remains constant after repeated recrystallization. Analysis gave:

I. 0.2173 gram substance dried at 105° gave 0.1603 gram H_2O and 0.3204 gram CO_2.

II. 0.1767 gram substance dried at 105° gave 24.5 cc. moist nitrogen at 16° and 746.7 mm.

	Calculated for $C_3H_7NO_2$.	Found. I.	Found. II.
C	40.44	40.26	...
H	7.87	8.19	...
N	15.73	...	15.93

As Hoogewerf and Van Dorp give for β-amidopropionic acid the melting-point 196°, being 16° higher than that found by Mulder,[3] we prepared some of the acid by their method. It was identical with that described above, melting with decomposition at 206°. To prepare large quantities of the acid it was found convenient to modify Hoogewerf and Van Dorp's method. 20 grams succinimide is added to a solution of 32 grams bromine in 900 grams of

[1] Recueil trav. chim. **10**, 5. [2] Heintz: Ann. Chem. (Liebig) **156**, 36.
[3] Ber. d. chem. Ges. **9**, 1903.

a 10-per cent. solution of potassic hydrate, the liquid slowly heated to 60° and kept at that temperature for two hours. To the cooled solution hydrochloric acid is added in excess, and the whole evaporated to dryness on the water-bath. The residue is exhausted with absolute alcohol (best in Soxhlet's apparatus), dry hydrochloric acid passed in, allowed to stand over night, decanted from sodic chloride, and evaporated to a syrup. This consists mainly of ethyl succinate and the hydrochloride of ethyl β-amidopropionate, and usually crystallizes on standing. It is dissolved in boiling water, a hot solution of baric hydrate added, boiled for half an hour and filtered. The barium is carefully precipitated with sulphuric acid, and the solution, after concentration, treated with lead oxide as above. The acid thus obtained is washed with hot absolute alcohol to remove any succinic acid that remains. The yield is about 65 per cent. of the theoretical.

It became necessary in the course of our work to prepare a number of simple derivatives of β-amidopropionic acid not hitherto described.

Sulphate of β-amidopropionic acid.—On evaporating a solution containing equivalent quantities of β-amidopropionic and sulphuric acids, fine colorless crystals of this salt remain. It may be purified by washing first with very little water and then with absolute alcohol. It melts with some decomposition at 150°, is easily soluble in water, difficultly in alcohol, and under the microscope is seen to crystallize in short, thick prisms. It does not lose in weight at 100°. Analysis gave: 0.4117 gram substance gave 0.345 gram $BaSO_4$.

	Calculated for $(C_3H_7NO_2)_2H_2SO_4$.	Found.
H_2SO_4	35.50	35.25

Hydrochloride of methyl β-amidopropionate, $HCl.NH_2.CH_2.CH_2.COOCH_3$.—β-Amidopropionic acid was allowed to stand twenty-four hours in a saturated solution of hydrochloric acid in methyl alcohol; the solution was then evaporated and the residue purified by fractional precipitation from chloroform by ligroïn and from methyl alcohol by ether. The salt forms white shining scales, which are hygroscopic and melt at 94°–95°.

0.1785 gram substance gave 16.5 cc. moist nitrogen at 24.5° and 731 mm.

	Calculated for $C_4H_9O_2N.HCl$.	Found.
N	10.03	10.00

Hydrochloride of ethyl β-amidopropionate, $HCl.NH_2.CH_2.CH_2.COOC_2H_5$, was first prepared and analyzed by Hoogewerff and Van Dorp, who, however, do not give its melting-point. We obtained it first on recrystallizing from boiling alcohol impure hydrochloride and amidopropionic acid formed by the decomposition of methyl carbomethoxyamidopropionate by hydrochloric acid. The hydrochloric acid had not been entirely removed and consequently etherification took place. Owing to the lack of data for identifying it, analyses were made.

I. 0.1632 gram substance gave 13.0 cc. moist nitrogen at 12° and 728 mm.

II. 0.0956 gram substance gave 0.0897 gram AgCl.

III. 0.2554 gram substance gave 0.188 gram H_2O, (CO_2 lost).

	Calculated for $C_5H_{11}O_2N.HCl$.	Found.
H	7.82	8.18
N	9.12	9.12
Cl	23.13	23.20

It was also prepared in the same way as the methyl ether. It crystallizes in white plates melting at 65.5°, and is easily soluble in water, alcohol and chloroform.

Synthesis of methyl carbomethoxy-β-amidopropionate.—β-Amidopropionic acid, probably on account of its salt-like nature, does not react at ordinary temperatures with methyl chlorformate. Its amine-like ethers and alkali salts react with great ease.

5.58 grams (2 mol.) of the hydrochloride $CH_3OOC.CH_2.CH_2.NH_2.HCl$ dissolved in 75 cc. chloroform was shaken for ten to fifteen minutes with 4.65 grams (1 mol.) of dried silver oxide. To the filtrate 1.95 grams (1 mol.) of methyl chlorformate dissolved in 50 cc. absolute ether was added. Some hydrochloride of methyl β-amidopropionate was precipitated almost immediately. More ether was added and the hydrochloride filtered off. On evaporating the filtrate, an oil remained which distilled without decomposition at 140° under 15 mm. pressure, became solid on cooling and then melted at 33.5°. All its properties are those of methyl carbomethoxy-β-amidopropionate obtained from succinimide bromide. Like it, it gives when shaken with lime-water an acid melting at 77°; with ammonia, the amide described below, melting at 142°. The reaction is

$$2CH_3OOC.CH_2.CH_2.NH_2 + Cl.CO.OCH_3 = CH_3OOC.CH_2.CH_2.NH_2.HCl + CH_3OOC.CH_2.CH_2.NH.COOCH_3.$$

The ether was also obtained from its synthetically-prepared acid.

Carbomethoxy-β-amidopropionic acid, $\begin{matrix} CH_2.NH.COOCH_3 \\ | \\ CH_2.COOH, \end{matrix}$

obtained from its ether by saponification, has already been described by us.[1] Its melting-point is 77°–77.5°. It may be obtained directly from potassium amidopropionate and methyl chlorformate:

$$2KOOC.CH_2.CH_2.NH_2 + Cl.CO.OCH_3 = \begin{matrix} CH_2.NH.COOCH_3 \\ | \\ CH_2.COOK \end{matrix} + KCl + NH_2.CH_2.CH_2.COOH.$$

1.9 grams amidopropionic acid were dissolved in a solution of 1.2 grams potassic hydrate in 3.5 cc. water and 1 gram methyl chlorformate added. The reaction was instantaneous, the mixture becoming quite warm. After diluting and washing with chloroform, an excess of hydrochloric acid was added and the mixture exhausted with chloroform. The product, after recrystallization, melts at 77° and is identical with the acid previously described. It is quite a stable compound, being one of the products of the action of potassic hydrate and concentrated hydrochloric acid on the ether. It is monobasic and yields corresponding salts.

Barium carbomethoxy-β-amidopropionate, $(CH_3.OOC.NH.CH_2.CH_2.COO)_2Ba$.—1.5 grams of the acid were dissolved in a little water and 1.5 grams (an excess) of barium carbonate added. When no more carbon dioxide was given off the solution was filtered, placed in a vacuum for a day, again filtered from a trace of baric carbonate, and left to evaporate over sulphuric acid. A sticky mass remained which soon became solid. It was powdered, washed with chloroform and dried over phosphoric anhydride. It is very soluble in water and melts with decomposition at 90°.

0.4224 gram substance gave 0.192 gram $BaCO_3$.

	Calculated for $(C_4H_6NO_4)_2Ba$.	Found.
Ba	31.93	31.61

The silver salt, $CH_3OOC.NH.CH_2.CH_2.COOAg$, prepared from the barium salt, is a curdy white precipitate which becomes dark on exposure to light and is sparingly soluble in water. For

[1] This Journal **15**, 219.

analysis it was dried on a clay plate and then in a desiccator in a dark place.

0.1709 gram substance gave 0.073 gram Ag.

	Calculated for $C_5H_9NO_4Ag$.	Found.
Ag	42.54	42.71

Ethyl carbomethoxy-β-amidopropionate, $CH_3.OOC.NH.CH_2.CH_2.COOC_2H_5$.—9 grams of acid were dissolved in 45 grams of alcohol. Hydrochloric acid was then passed in, and, after standing, the alcohol evaporated. The residue was purified by dissolving in chloroform and precipitating with ligroïn. Most of the dark oil precipitated distilled at 135°–137°, under 14 mm. pressure, as a colorless oil which solidified in a freezing-mixture and melted at 15.5°. Shaken with milk of lime it yields carbomethoxyamidopropionic acid. Analyses:

I. 0.2663 gram substance gave 0.1775 gram H_2O and 0.4634 gram CO_2.

II. 0.1767 gram gave 12.5 cc. moist nitrogen at 755.5 mm. and 16.5°.

	Calculated for $C_7H_{13}NO_4$.	Found.
H	7.43	7.82
C	48.00	47.46
N	8.00	8.21

Carbomethoxy-β-amidopropionamide, $CH_3OOC.NH.CH_2.CH_2.CONH_2$.—2 grams of methyl carbomethoxyamidopropionate were warmed with about 12 cc. concentrated aqueous ammonia to 55°–60° for 15 minutes, and the solution then allowed to evaporate in a vacuum-desiccator. Colorless crystals remained, which were dried on a clay plate, washed with ether, and dissolved in a boiling mixture of equal volumes of absolute alcohol and chloroform. On adding a second volume of chloroform and sufficient ligroïn, the amide was precipitated in microscopic needles. From water or ammonia it crystallizes in broad orthorhombic plates with sharply defined lines of cleavage. Large crystals of the amide are deposited when the ether is dissolved in five times its weight of concentrated ammonia solution and allowed to stand a few days. The amide melts at 142.5°. Analyses:

I. 0.200 gram substance, dried at 100°, gave 0.1284 gram H_2O and 0.3014 gram CO_2.

II. 0.1242 gram substance gave 21 cc. moist nitrogen at 20.5° and 739.7 mm.

	Calculated for $C_5H_{10}N_2O_3$.	Found.
C	41.10	41.10
H	6.85	7.13
N	19.18	19.06

The well-known stability of the urethanes and the extreme instability of the carbamic acids indicated almost beyond a doubt that the carboxyl of the acid $C_5H_9O_4N$ is not attached to nitrogen and that therefore its constitution is that given, *viz.* $\begin{matrix}CH_2.NH.COOCH_3\\ |\\ CH_2.COOH\end{matrix}$.

This is shown directly by the behavior of the mixed ethers.

Methyl carbethoxy-β-amidopropionate, $\begin{matrix}CH_2NH.COOC_2H_5\\ |\\ CH_2.COOCH_3\end{matrix}$.—12.9 grams of the hydrochloride of methyl amidopropionate were treated[1] with 10.7 grams silver oxide and 5 grams ethyl chlorformate:

$$2CH_3OOC.CH_2.CH_2.NH_2 + Cl.CO.OC_2H_5 =$$

$$CH_3OOC.CH_2.CH_2.NH_2.HCl + \begin{matrix}CH_2.NH.COOC_2H_5\\ |\\ CH_2.COOCH_3\end{matrix}.$$

The oily product distilled at 134°–137° under 15 mm. pressure, and then solidified in a freezing-mixture. It melts below 0°. 0.204 gram substance gave 14.5 cc. moist nitrogen at 18.5° and 733 mm.

	Calculated for $C_7H_{13}NO_4$.	Found.
N	8.0	7.89

On being saponified with lime-water, the methyl group is split off and

Carbethoxy-β-amidopropionic acid, $C_2H_5OOC.NH.CH_2.CH_2.COOH$, is formed. It was obtained as an oil, which solidified in the cold, and was purified by dissolving in chloroform and precipitating with ligroïn. It forms colorless crystals melting at 59°.

0.2221 gram substance gave 17.4 cc. moist nitrogen at 20.5° and 733.5 mm.

	Calculated for $C_6H_{11}NO_4$.	Found.
N	8.70	8.66

Carbethoxy-β-amidopropionamide, $C_2H_5OOC.NH.CH_2.CH_2.CO.NH_2$.—1 gram of the ether was mixed with 7 grams concentrated aqueous ammonia, heated to 60° and evaporated over sulphuric

[1] See page 510.

acid. Recrystallized by precipitating with ligroïn from a mixture of alcohol and chloroform, it forms colorless crystals which melt at 120.5°.

0.1607 gram substance gave 23.9 cc. moist nitrogen at 10° and 745 mm.

	Calculated for $C_8H_{12}N_2O_5$.	Found.
N	17.50	17.49

These results afford absolute proof that in the ether

$$\begin{array}{l} CH_2.NH.COOCH_3 \quad (I) \\ | \\ CH_2.COOCH_3 \quad (II) \end{array},$$

it is the group $COOCH_3$ (II) that is reactive.

Dimethyl succinylo-β-ureidopropionate,

$$\begin{array}{ll} CH_2.NH.CO.NH.CH_3 & \\ | & | \\ CH_2.COOCH_3 & CH_2.COOCH_3, \end{array}$$

is formed when sodium methylate acts on succinimide bromide in concentrated methyl-alcoholic solution. It has already been described by us.[1] It melts at 65.5°. By mild saponification it yields succinic acid and β-ureidopropionic acid. 3 grams of the ether were shaken with milk of lime at intervals for three hours, the solution filtered, nearly all the calcium precipitated with oxalic acid, and the filtrate evaporated at 50° under diminished pressure. The residue, which was washed with alcohol and dried on a clay plate, proved to be β-ureidopropionic acid.[2] The alcoholic washings left on evaporation succinic acid.

As it is extremely improbable that so mild an alkali causes a molecular rearrangement, the above decomposition must be:

$$\begin{array}{ll} CH_2.NH.CO.NH.CO.CH_2 & \\ | & | \\ CH_2.COOCH_3 & CH_2.COOCH_3 \end{array} + 3H_2O =$$

$$\begin{array}{ll} CH_2.NH.CO.NH_2 & HOOC.CH_2 \\ | & | \\ CH_2.COOH & HOOC.CH_2 \end{array} + 2CH_3OH,$$

and the constitution of the compound must be that given.

Decomposed with concentrated hydrochloric acid, dimethyl succinylo-β-ureidopropionate yields succinic acid and β-lactylurea, hydrochloric acid readily condensing β-ureidopropionic acid (see below):

[1] This Journal **15**, 220.

[2] See page 515.

$$\begin{array}{l} CH_2.NH.CO.NH.CO.CH_3 \\ | \qquad\qquad\qquad\quad | \\ CH_2.COOCH_3 \qquad CH_2.COOCH_3 \end{array} + 2H_2O =$$

$$\begin{array}{l} CH_2.NH.CO \qquad CH_2.COOH \\ | \qquad | \qquad + \quad | \\ CH_2.CO.NH \qquad CH_2.COOH \end{array} + 2CH_3OH.$$

The reaction has been described by us.[1] The β-ureidopropionic acid and β-lactylurea thus obtained are identical with preparations which we have made synthetically.

Methyl β-ureidopropionate, $NH_2.CO.NH.CH_2.CH_2.COOCH_3$. —To a cold solution of 2.5 grams (1 mol.) of the hydrochloride of methyl amidopropionate 1.5 grams (1 mol.) of potassium cyanate are added, and the mixture slowly evaporated on a water-bath. The powdered residue is washed with ether and then exhausted with hot chloroform. The chloroform gives on evaporation a syrup, which soon crystallizes. From benzene it was precipitated by ligroïn as a colorless oil which crystallizes on standing in silky needles, which under a microscope are seen to be long, flat prisms. It melts at 66.5°, is easily soluble in water, alcohol and chloroform, difficultly in ether, ligroïn, and benzene even when warm.

0.1279 gram substance gave 22.7 cc. moist nitrogen at 23.5° and 733.2 mm.

	Calculated for $C_5H_{10}N_2O_3$.	Found.
N	19.18	19.27

It is isomeric with carbomethoxy-β-amidopropionamide, described above.

β-Ureidopropionic acid, $NH_2.CO.NH.CH_2.CH_2.COOH$. — 3 grams of methyl β-ureidopropionate were allowed to stand for 6 hours with 5 grams (an excess) of baric hydrate in 100 cc. of water. The barium, with the exception of a trace, was precipitated with sulphuric acid, and the filtrate evaporated at 40°–50° under diminished pressure. Three crops of crystals were obtained, of which the first contained some β-lactylurea, the third a little barium salt. The intermediate product was pure acid; it was brought on a clay plate and dried in a desiccator.

0.1045 gram substance gave 19.5 cc. moist nitrogen at 21° and 741.8 mm.

	Calculated for $C_4H_8N_2O_3$.	Found.
N	21.21	20.97

[1] This Journal **15**, 221.

It is not easily soluble in cold water, difficultly in organic solvents, and dissolves readily in hot water, the alkalies, and alkali carbonates. It melts with evolution of gas at 170°–171°. It is very unstable, readily losing water and forming β-lactylurea. This takes place even when its aqueous solution is evaporated on a water-bath with some hydrochloric acid. On heating 10 to 15 minutes, first at 160° and then at 170°, condensation takes place almost quantitatively.

$$\begin{matrix} CH_2.NH.CO.NH_2 \\ | \\ CH_2.COOH \end{matrix} = \begin{matrix} CH_2.NH.CO \\ | \quad | \\ CH_2.CO.NH \end{matrix} + H_2O.$$

Unlike the sulphates of other amido-acids,[1] β-amidopropionic acid sulphate does not react smoothly with potassium cyanate. On mixing solutions of the two, effervescence and the odor of cyanic acid were observed, and no ureidopropionic acid or lactylurea was found in the product obtained. This behavior seems to be due to the smaller influence of the amido-group in the β- than in the α-position on the acid radicle, so that the weaker cyanic acid cannot combine with the base. For this reason the methyl ether of the acid was used instead, as above. It was afterwards found that β-amidopropionic acid itself reacts with the greatest ease with potassium cyanate, forming the salt $\begin{matrix} CH_2.NH_2.(HNCO) \\ | \\ CH_2.COOK \end{matrix}$. This, when its solution is treated with acids, gives off cyanic acid. On evaporating its aqueous solution it is converted into

Potassium β-ureidopropionate, $NH_2.CO.NH.CH_2.CH_2.COOK$. —Solutions of 2 grams (1 mol.) of β-amidopropionic acid and 1.85 grams (1 mol.) potassium cyanate were mixed and slowly evaporated on a water-bath. From the syrupy residue, on standing, large colorless crystals separated. They were removed from the mother-liquor, brought on a clay plate and immediately placed in a desiccator. The salt is extremely hygroscopic, softens at 80° and melts at 100°. Heated at 100°–105° for one hour, it lost less than 1 per cent. in weight.

0.146 gram substance, dried at 105°, gave 0.0743 gram K_2SO_4.

	Calculated for $C_4H_7KN_2O_3$.	Found.
K	22.93	22.85

[1] Menschutkin: Ann. Chem. (Liebig) **153**, 83; etc.

Evaporated on the water-bath with dilute hydrochloric acid, it is converted quantitatively into β-lactylurea. This method of obtaining β-ureidopropionic acid and β-lactylurea from the amido-acid and potassium cyanate gave a very good yield and suggests that it may be extensively used with advantage for this purpose. β-ureidopropionic acid is also one of the products of the action of mild alkalies on dimethyl succinyloureidopropionate.

β-Lactylurea, $\begin{matrix} CH_2.NH.CO \\ | \quad\quad | \\ CH_2.CO.NH \end{matrix}$, as obtained from the ureido-acid, was purified by recrystallizing from hot 50-per cent. alcohol. Dried at 100°, it fused at 272° with decomposition, and proved identical in all respects with the product obtained from dimethyl succinyloureidopropionate and already described.[1]

0.1128 gram synthetically-prepared substance gave 23.8 cc. moist nitrogen at 12° and 745.2 mm.

	Calculated for $C_4H_6N_2O_2$.	Found.
N	24.56	24.64

It has a neutral reaction and is insoluble in sodic carbonate, but dissolves readily in caustic alkali, like other ureïdes. Its silver salt,

Silver β-lactylurea, $\begin{matrix} CH_2.NH.CO \\ | \quad\quad | \\ CH_2.CO.N.Ag \end{matrix}$, was prepared by dissolving 0.8 gram (a slight excess) substance in 3.6 cc. of a 10-per cent. solution of potassic hydrate, filtering from a slight undissolved residue, and adding a concentrated solution of 1.2 grams silver nitrate. The heavy white curdy precipitate was washed with a little cold water, brought on a clay plate and dried over sulphuric acid in a dark place. It becomes slightly darker on exposure to light, and is somewhat soluble in cold water.

0.3186 gram substance gave 0.1545 gram Ag.

	Calculated for $C_4H_5AgN_2O_2$.	Calculated for $C_4H_7AgN_2O_3$.[2]	Found.
Ag	48.87	45.19	48.49

As the molecular rearrangement of the nitrogen derivatives

$$-C=N$$
$$\quad | \quad | \quad$$
$$\quad Y \quad X$$

[1] This Journal 15, 221.

[2] $NH_2.CO.NH.CH_2.CH_2.COOAg$.

is supposed to depend on the relative negative and positive character of the groups X and Y, we propose to vary group Y, so as to obtain, if possible, substances in which the rearrangement does not occur, and to use other nitrogen halogen compounds for the same purpose.

Contributions from the Chemical Laboratory of Cornell University.

ON THE DECOMPOSITION OF DIAZOBENZENE SULPHATE IN ISOAMYL ALCOHOL.

By W. R. Orndorff and C. G. Hopkins.

It has been shown by Remsen and Orndorff[1] that when diazobenzene sulphate is decomposed by heating it in absolute ethyl alcohol, two reactions take place simultaneously, yielding phenylethyl ether in the first case, and benzene in the second. The normal reaction appears to be the one forming phenylethyl ether according to the equation

$$C_6H_5.N_2.HSO_4 + HOC_2H_5 = C_6H_5.OC_2H_5 + H_2SO_4 + N_2.$$

The second reaction, yielding benzene, takes place according to the equation

$$C_6H_5.N_2.HSO_4 + C_2H_6O = C_6H_6 + N_2 + H_2SO_4 + C_2H_4O,$$

and appears to be subsidiary to the first, as the yield of phenylethyl ether is much greater than that of benzene.

Quite recently Parks[2] has shown that when paradiazoorthotoluenesulphonic acid is decomposed with methyl alcohol, paramethoxyorthotoluenesulphonic acid is the only product of the reaction. The second reaction, which we should expect to yield orthotoluenesulphonic acid, does not take place here. When paradiazoorthotoluenesulphonic acid is decomposed in ethyl alcohol, Remsen and Palmer[3] have shown that the two reactions take place simultaneously, yielding both ethoxytoluenesulphonic acid and toluenesulphonic acid, although the amount of the latter was small in comparison with the amount of the former. Thinking that the difference in the conduct of this diazo

[1] This Journal **9**, 387. [2] *Ibid.* **15**, 320. [3] *Ibid.* **8**, 243.

compound when decomposed in methyl and ethyl alcohols might be due to differences in the two alcohols, the following experiments on the decomposition of diazobenzene sulphate in isoamyl alcohol were undertaken by the authors. It was thought that, since the diazo compounds when decomposed with methyl alcohol gave only the methoxy compound, while, when decomposed with ethyl alcohol, both reactions take place, yielding the ethoxy compound as well as the sulphonic acid; that if higher alcohols of the methyl alcohol series were used, the second reaction—the so-called hydrogen reaction—would alone take place. Unfortunately this has not proven to be the case; yet as a new reaction of the diazo compounds was discovered in the course of this work, these results are here recorded.

The diazobenzene sulphate was made by the method of Knoevenagel[1] and decomposed in absolute isoamyl alcohol (boiling at 130°–131°) in portions of 20 grams. The dry diazobenzene sulphate, together with about three times its weight of isoamyl alcohol, was placed in a round-bottomed flask which was connected with a return-condenser and heated on a water-bath. About 400 grams of the diazobenzene sulphate were thus decomposed. The decomposition took place rapidly and smoothly below the boiling-point of water, generally beginning at 70°. After the decomposition had apparently ceased the heat was increased until the water in the bath was boiling. Gas was given off freely during the decomposition, and in several cases this gas smelled strongly of benzene. Some of this gas was collected and carefully examined. It was found to be pure nitrogen containing a very small amount of isoamyl-alcohol and benzene vapors. The flask containing the diazo compound and the isoamyl alcohol was weighed before and after the decomposition, and the loss in weight due to the escaping gas was found in every case to agree very well with that required by the theory. During the decomposition of the diazo compound the isoamyl alcohol becomes dark red in color, probably owing to the formation of some azo dyestuff.

After the decomposition of the diazo compound was completed the separate portions were united and treated with caustic-soda solution to remove the sulphuric acid, preliminary experiments having shown that unless this was done the sulphuric acid would act on the isoamyl alcohol, forming secondary products, such as

[1] Ber. d. chem. Ges. **23**, 2994.

amylene and diamylene. The isoamyl alcohol solution of the decomposition-products of the diazobenzene sulphate was then subjected to fractional distillation through a Hempel tube. The distillate separated into two layers, an upper one consisting largely of isoamyl alcohol and a lower one which was nearly all water. On redistilling this aqueous layer through a Hempel distilling-tube a liquid was obtained boiling at 92°–93°, which had the odor of an aldehyde and whose solution in water reduced an ammoniacal solution of silver nitrate very readily. This liquid was identified as isovaleric aldehyde.

The upper layer of the first distillate was also redistilled through a Hempel distilling-tube. A product was obtained which boiled below 90° and which was shown to be benzene by its boiling-point, physical properties and ready formation of dinitrobenzene when treated with a mixture of nitric and sulphuric acids.

Only a few cubic centimeters of the benzene and the isovaleric aldehyde were obtained and it is quite evident that they are not the chief products of the reaction.

Besides the benzene and the isoamyl alcohol the upper layer gave two other products when refractioned several times, one boiling at 183°–184° (uncorr.) and which solidified when cooled with ice-water, and the other boiling at 215°–220° (uncorr.). The liquid boiling at 183°–184° was formed in large quantity and was the principal product of the decomposition. It melted at about 30°, after it had been once solidified and separated from the oil which accompanied it, and gave all the reactions for phenol. After being carefully purified by redistillation and recrystallization it was analyzed. The results follow:

I. 0.4664 gram substance gave 1.3110 grams CO_2 and 0.2930 gram H_2O.

II. 0.4247 gram substance gave 1.1910 grams CO_2 and 0.2780 gram H_2O.

	I.	II.	Theory for C_4H_4O.
C	76.66	76.48	76.60
H	6.98	7.27	6.38
O (by difference)	16.36	16.25	17.02

Vapor-density determinations by the Victor Meyer method gave the following results:

	I.	II.
Weight of substance	0.154 gram.	0.1125 gram.
Barometer	740 mm.	740 mm.
Volume of air expelled	39.8 cc.	29.7 cc.
Temperature of air when measured	21° C.	20.5°.

	I.	II.	Theory for C_6H_6O.
Vapor-density found (referred to air)	3.39	3.31	3.26

From these results and the properties of the substance it was concluded that the chief product of the reaction is undoubtedly phenol.

The product boiling at 215°–220° was also purified by redistillation and analyzed. The results follow:

I. 0.2188 gram substance gave 0.6404 gram CO_2 and 0.1889 gram H_2O.

II. 0.2548 gram substance gave 0.7436 gram CO_2 and 0.2204 gram H_2O.

	I.	II.	Theory for $C_6H_5.O.C_5H_{11}$.
C	79.82	79.59	80.50
H	9.59	9.61	9.80
O (by difference)	10.59	10.80	9.70

A vapor-density determination by the Victor Meyer method gave the following result:

Weight of substance	0.1335 gram.
Barometer	740 mm.
Volume of air expelled	20.7 cc.
Temperature of air when measured	21°

		Theory for $C_6H_5.O.C_5H_{11}$.
Vapor-density found (referred to air)	5.66	5.67

These results show that the substance is isoamylphenyl ether. The amount of this substance formed was very small.

The products of the decomposition of diazobenzene sulphate in isoamyl alcohol are, then, phenol, isoamylphenyl ether, benzene, isovaleric aldehyde, nitrogen and sulphuric acid. The isoamylphenyl ether is formed by the decomposition of the diazobenzene sulphate according to the equation

$$C_6H_5.N_2|HSO_4 + H|OC_5H_{11} = C_6H_5.O.C_5H_{11} + N_2 + H_2SO_4,$$

free nitrogen and sulphuric acid being formed at the same time.

The benzene and isovaleric aldehyde result from the decomposition of the diazo salt as follows:

$$C_6H_5.N_2.HSO_4 + C_5H_{12}O = C_6H_6 + C_5H_{10}O + H_2SO_4 + N_2.$$

Regarding the formation of phenol, the authors believe that the decomposition may be correctly represented by the following equation:

$$C_6H_5.N_2|HSO_4 + C_5H_{11}|OH = C_6H_5OH + C_5H_{11}.HSO_4 + N_2,$$

the products being phenol, isoamyl acid sulphate and free nitrogen. In support of this view of the formation of phenol it may be stated that the isoamyl alcohol used in the decompositions boiled constantly at 130°–131° and was shown to be absolutely anhydrous. The diazobenzene sulphate was dry and crystalline. It was washed with alcohol and then with ether before drying, so that it may be assumed that it was free from water. Isoamyl acid sulphate was found among the products of the reaction, but unfortunately it could not be determined whether it resulted from this reaction or from the action of the sulphuric acid, set free in the other reactions, on the isoamyl alcohol. There seems, however, to be no good reason why sulphuric acid should be formed here in preference to isoamyl acid sulphate, and as the formation of the latter explains in a satisfactory manner the production of phenol, it has been assumed here.

Experiments were then undertaken upon the decomposition of the diazo compound obtained from metanitraniline. The metanitrodiazobenzene sulphate was made by the method of Knoevenagel and decomposed in isoamyl alcohol, according to the directions given above. Nitrobenzene, isovaleric aldehyde and nitrogen were formed in the decomposition and were readily identified, but no metanitrophenol could be isolated from the products. A very large quantity of a brown dyestuff was formed which was shown to be metadinitrooxyazobenzene, $C_6H_4(NO_2).N_2.C_6H_4(OH).NO_2$. This was probably formed by the metanitrophenol combining as soon as formed with metanitrodiazobenzene sulphate according to the equation:

$$C_6H_4\begin{cases}NO_2\\N_2.HSO_4\end{cases} + C_6H_4\begin{cases}NO_2\\OH\end{cases} =$$

$$C_6H_4\begin{cases}NO_2(m)\\N_2C_6H_3\end{cases}\begin{cases}NO_2\\OH(m)\end{cases} + H_2SO_4.$$

The metanitrophenol itself being formed according to the reaction represented by the equation

$$C_6H_4\langle^{NO_3}_{N_2}|HSO_4 + C_5H_{11}|OH = C_6H_4\langle^{NO_2}_{OH(m)} + C_5H_{11}HSO_4 + N_2.$$

Further investigation of this interesting reaction is in progress.

ITHACA, *May*, 1893.

Contribution from the Chemical Laboratory of Clark University.

A NEW CLASS OF ORGANIC ELECTROSYNTHESES.

BY S. P. MULLIKEN.

Although the number of organic compounds that have been subjected to electrolysis is large, the organic acids containing the carboxyl group together with their salts remain the only class in regard to whose electrolytic decomposition-products generalizations of much interest have been reached. The products obtained on passing the electric current through solutions of other organic substances have for the most part owed their origin to secondary reactions,—generally oxidations or reductions. Such secondary reactions were invariably observed by the earlier experimenters in this field, when the futile attempt was made to confer the property of electrical conductivity on a non-electrolyte by adding to it a mineral acid or base. Thus it was early discovered that the electrolysis of alcohol containing sulphuric acid yields aldehyde, formed by the action on the alcohol of the electrolytic oxygen liberated, while nitrobenzene mixed with weak alkaline or saline solutions is so readily reduced by electrolytic hydrogen that the electrolysis of such a mixture is a process that has been patented.[1]

This article contains the first results obtained in a series of electrosynthetic experiments still in progress, which were begun by the author while a fellow of Clark University, more than two years ago. These experiments were undertaken with a view to deter-

[1] Ber. d. chem. Ges. **16**, 96.

mine whether certain organic compounds not belonging to the carboxylic acid or salt type, possessing acid properties, but not generally recognized as electrolytes, could be electrolyzed; and if so, whether the negative ions liberated from them could be caused to unite with one another by employing methods similar to those used by Crum-Brown and Walker in their interesting syntheses of dibasic acids from sodic ethylic malonate and its homologues.[1]

The group of substances possessing these acidic properties is a large and heterogeneous one. Among its members may be mentioned ketonic derivatives like acetylacetone and acetoacetic ether; sodium salts, like that of malonic ether; imides, and nitro-paraffin derivatives. These compounds are all exceedingly weak acids and their salts are unstable. Acetylacetone, which is one of the strongest acids of the group, expels carbon dioxide from carbonates. Acetoacetic and ethylacetoacetic ethers are, however, so weak that, according to the recent molecular-conductivity measurements of Walden, even acetic acid is by comparison an excellent conductor:[2]

Acetic acid.		Acetoacetic ether.		Ethylacetoacetic ether.	
v.	μ.	v.	μ.	v.	μ.
128	16.99	128	1.18	128	0.068
256	23.82	256	1.20	256	0.070

v = dilution in litres per gram-equivalent.
μ = molecular conductivity.

Diethylic malonate, $CH_2(CO_2C_2H_5)_2$, does not conduct electricity at all, and it was only by using its sodium salt, which is probably represented[3] by the symbol $\begin{matrix} C<^{OC_2H_5}_{ONa} \\ \| \\ HC.CO_2C_2H_5 \end{matrix}$, that it became possible to employ it in electrolytic experiments.

The products obtained in all the electrosyntheses thus far made are the same that one would expect to be formed by the action of iodine on the sodium or silver compounds of the substances electrolyzed. Thus:

$$2C_7H_{11}O_4Na + 2I = 2NaI + C_2H_2(CO_2C_2H_5)_4, \text{ and}$$
Sodium diethylic malonate. Ethylic ethanetetracarboxylate.

$$2C_7H_{11}O_4Na \text{ (electrolyzed)} = 2Na + C_2H_2(CO_2C_2H_5)_4.$$

[1] Ann. Chem. (Liebig) **261**, 107. [2] Walden: Ber. d. chem. Ges. **24**, 2025.
[3] Nef: Ann. Chem. (Liebig) **266**, 114.

The yields from the iodine reaction, when this could be used, were generally better than those given by the electrical method. In the synthesis of ethylic ethanehexacarboxylate, however, a fair yield of an interesting body was obtained by the electrical method, when the iodine reaction signally failed. The two methods are therefore to be regarded as supplementary to each other, and it is to be hoped that a careful study of the conditions favorable to electrosynthesis of this kind will result in improvements in the electrical method that will lead to larger yields.

The electrical apparatus for the early experiments was simple, but not altogether satisfactory. The electrolytic cells were thin-walled glass cylinders 3–5 cm. in diameter, having capacities of from 30–100 cc. The anodes were spirals of platinum wire 1–2 mm. in diameter, and the cathodes rectangular strips of platinum foil almost wide enough, when folded into cylinders, completely to surround the spiral anodes. Accidental contacts between the electrodes were provided against by inserting a narrow glass ring between them at the bottom of each cell. The wires connected with the electrodes passed through perforations in the corks closing the cylinders. The necessarily high electrical resistance offered by the solutions always caused some heating. Each cylinder was therefore surrounded by a cooler through which water circulated. When the quantity of a solution was small, or the resistance very high, the capacity of a cell was diminished and the electrodes brought nearer together by winding the platinum spiral about a stout glass cylinder closed at its lower end. With this arrangement, by establishing a circulation of cold water within the inner cylinder, an effective internal cooler could also be improvised. The use of a platinum spiral having a comparatively small surface as anode—only about one-tenth of the surface of the cathode—is a device of Crum-Brown and Walker's for increasing the density of the current at the anode in order to give the most favorable conditions for the union to molecules of the nascent radicles there liberated.

The current was furnished by a battery of 49 "crowfoot" gravity-elements set up in series. This form of battery was employed because a dynamo and storage cells were not available, and a constant current could be obtained from it for weeks together without requiring much attention to keep it in order. Its internal resistance was very high (often two ohms to the cell), so that the

current obtainable from it was always weak—a disadvantage which was partly remedied by multiplying the number of electrolytic cells and connecting them in parallel circuit.

Electrolysis of Sodic Diethylic Malonate (*Ethylic Ethanehexacarboxylate*).—$2\,C<^{OC_2H_5}_{ONa}\,\|\,HC.CO_2C_2H_5 = Na_2 + 2\,CH(CO_2C_2H_5) \rightarrow CH.(CO_2C_2H_5)_2\,|\,CH.(CO_2C_2H_5)_2$.

This electrolysis was performed in an alcoholic solution. To prevent the accumulation of uncombined sodium ethylate during the passage of the current, this solution was prepared so as to contain malonic ether as well as its sodium compound. The sodium set free at the cathode reacted on the free malonic ether present to form a fresh quantity of sodium salt, and the strength of the electrolyte was thus maintained constant for a long time.

3 grams of sodium were dissolved in 40 grams of well-cooled malonic ether and the solution made up to 100 cc. by adding absolute alcohol. A current of one-sixth of an ampère was then passed through the solution for 23 hours. Hydrogen was disengaged at the cathode, while little or no gas appeared at the other pole. The clear, yellowish solution was neutralized with acetic acid, evaporated on the water-bath, and the residue extracted with ether. An oil was thus obtained which fractionation under a pressure of 15 mm. showed to consist almost entirely of two constituents. One of these boiled between 90° and 100° and was malonic ether. The other boiled between 190° and 210° and solidified in a freezing-mixture. Its weight amounted to about 25 per cent. of that of the malonic ether present in solution as sodium salt at the beginning of the electrolysis. Crystallized from alcohol, this compound formed a compact mass of long, white needles, melting at 73°. According to an analysis it contained 52.69 per cent. of carbon and 6.53 per cent. of hydrogen. A comparison of its properties and appearance with those of a specimen of pure ethylic "acetylenetetracarboxylate" prepared by Bischoff's method[1] from iodine and the sodium salt of malonic ether, left no doubt that the two bodies were really identical. The body obtained by Conrad's method melted at 76° and should contain 52.83 per cent. of carbon and 6.92 per cent. of hydrogen. The slightly

[1] Bischoff, Rach: Ber. d. chem. Ges. **17**, 2781.

lower melting-point observed for the body obtained by the electrical method seemed due to a mere trace of some impurity that could not easily be removed by crystallization.

Electrolysis of Sodic Ethylic Methanetricarboxylate (*Ethylic Ethanehexacarboxylate*).—Ethylic methanetricarboxylate, $CH.(CO_2C_2H_5)_3$, and its sodium salt were prepared by the method described by Michael.[1] The sodium salt was used because the pure ester is such a poor conductor that an electrolysis of its solution was found impracticable. The following reaction may be assumed to take place when the current traverses solutions of the sodium salt:

$$2\begin{matrix} C<^{OC_2H_5}_{ONa} \\ \| \\ C(CO_2C_2H_5)_2 \end{matrix} = Na_2 + 2C(CO_2C_2H_5)_2 \rightarrow \begin{matrix} C-(CO_2C_2H_5)_3 \\ | \\ C-(CO_2C_2H_5)_3 \end{matrix}.$$

The sodium salt obtained by Michael's method is a white powder readily soluble in water and without decomposition—unlike the corresponding salt of malonic ether, which water immediately splits into sodic hydrate and malonic ether. The stability of such solutions is, however, only relative; for on standing for a few hours, even in closed vessels from which the air is excluded, all the ethylic methanetricarboxylate present separates out as an oily layer. Three portions of sodium salt weighing 15 grams each, were dissolved in 30 cc. of cold distilled water. Each portion was electrolyzed in a small cell provided with an efficient water-cooler. A current of one-third of an ampère was passed through the solutions for 24 hours, although a shorter electrolysis would doubtless have given equally good results, since after two or three hours all the ester that had at first been combined with sodium seemed to have separated out at the bottoms of the cells. Carbon dioxide was evolved at the anode, and a considerable quantity of acid sodic carbonate separated out with the oil. The alkaline solution above the oil possessed a very peculiar pungent odor.

The contents of the three cells were united, neutralized with dilute sulphuric acid and shaken out with ether. The oil dissolved by the ether was distilled under a pressure of 24 mm. Boiling began at 90° and was interrupted at 230°. The residue in the flask was an oily mass of the consistency of butter, which weighed 6 grams. The neck of the distilling-flask was dotted with small semi-spher-

[1] A. Michael: J. prakt. Chem. [2] **37**, 476.

ical aggregates of a crystalline solid, and a small quantity of this same substance separated from that portion of the distillate boiling between 190° and 230°, when this was cooled down to −10°. The lower-boiling fractions were mixtures of ethylic methanetricarboxylate and ethylic malonate. The ethylic malonate and acid sodic carbonate were probably formed by the following secondary reactions which took place in the cell during the electrolysis, and have necessarily no direct connection with the electrolytic process proper:

(*a*). $C_{10}H_{15}O_6Na + H_2O = C_{10}H_{16}O_6 + NaOH$.

(*b*). $C_{10}H_{16}O_6 + NaOH + H_2O =$

$$CH_2(CO_2C_2H_5)_2 + C_2H_5OH + NaHCO_3.$$

Reaction (*b*) has already been mentioned by Conrad and Guthzeit.[1] The solid compound present in the two highest-boiling fractions was purified by slow crystallization from ethereal solution. The hard, white crystalline crusts obtained were repeatedly washed with small quantities of dry ether. The compound melted at 101° (corrected). It is freely, but very slowly soluble in alcohol and in ether. Elementary analysis gave results agreeing with the numbers expressing the percentage-composition of the hexethylic ether of ethanehexacarboxylic acid.

I. 0.3186 gram substance gave 0.6062 gram CO_2 and 0.1844 gram H_2O.

II. 0.2468 gram substance gave 0.4676 gram CO_2 and 0.1432 gram H_2O.

	Calculated for $C_{20}H_{30}O_{12}$.	Found. I.	Found. II.
C	51.95	51.90	51.80
H	6.49	6.43	6.45
O	41.56	41.67	41.75
	100.00	100.00	100.00

The molecular weight of the ether determined by the freezing-point method was found to be 442; the molecular weight calculated for the compound $C_{20}H_{30}O_{12}$ is 461.

The constitution of this ether has been definitely proved to correspond to the formula above given to it, by experiments just completed in this laboratory by Mr. J. B. Weems, an account of which will soon appear in this Journal. With the exception of the butanehexacarboxylic ether of Bischoff and Rach[2] it is the first

[1] Ann. Chem. (Liebig) **214**, 34 and 37.

[2] Ber. d. chem. Ges. **17**, 2786.

derivative of a hexacarbonic acid that has been prepared in the aliphatic series.

Electrolysis of Acetylacetone (Tetracetylethane). — Unlike malonic ether which in alcoholic solution is a non-conductor, a solution of acetylacetone in dilute alcohol is a true electrolyte, although not a very good one. This electrolysis therefore differs from the two preceding in that the positive ion is hydrogen instead of a metal.

Combes has shown[1] that acetylacetone possesses such strong acid properties that it is able to expel carbonic acid from all the metallic carbonates, thus proving itself a much stronger acid than acetoacetic ether. This fact, together with the interesting results obtained by Perkin in a recent study of the molecular magnetic rotation of acetyl acetone, render it highly probable that this substance is really an unsaturated alcohol in which the number of hydroxyl groups and double bondings present in any one molecule is to a certain extent dependent upon the temperature.[2] At the ordinary temperature, according to Perkin, the compound $\begin{matrix}CH_3\\OH\end{matrix}\!>\!C=CH.C\!\begin{matrix}//O\\\backslash CH_3\end{matrix}$ predominates, and it is by the presence of such molecules, which are analogous in structure to the sodium compound of malonic ether, and might very naturally be dissociated to some extent in solution, that the formation of tetracetyl ethane as a product of the electrolysis of acetylacetone may be ascribed.

$$2\begin{matrix}CH_2\\OH\end{matrix}\!>\!C=CH.C\!\begin{matrix}//O\\\backslash CH_3\end{matrix}=H_2+\begin{matrix}CH_3\\OH\end{matrix}\!>\!C=C-C\!\begin{matrix}//O\\\backslash CH_3\end{matrix}$$
$$\begin{matrix}|\\CH_2\\OH\end{matrix}\!>\!C=C-C\!\begin{matrix}//O\\\backslash CH_3\end{matrix}$$

The above equation expresses what would be expected if the electrolysis of acetylacetone is a reaction that is strictly analogous to the two other reactions already described. The product would be a tetracetylethane that is at once alcohol and ketone, and which is isomeric with the tetraketone $(C_5H_8O)_2CH—CH(C_2H_3O)_2$, the compound that by a strict nomenclature should be called tetracetylethane.

40 grams of pure "acetylacetone" prepared by Claissen's method[3] were dissolved in 60 cc. of 50-per cent. alcohol and the solution

[1] A. Combes: Compt. Rend. **105**, 868. [2] Perkin: Jour. Chem. Soc. 1892, 816.
[3] Ber. d. chem. Ges. **22**, 1009.

distributed among three small electrolytic cells connected in parallel circuit. The electrolysis was continued for three weeks. The current which passed through all these cells during this period averaged about one-fifteenth of an ampère.

Hydrogen was continuously evolved at the cathode, and white crystalline scales were noticed at the bottom of one cell as early as the end of the first day. These crystals, although freely soluble in boiling alcohol, were very sparingly soluble in cold alcohol. Had the compound been more soluble in cold dilute alcohol its formation might have escaped observation. The crystals were removed from the cells every three or four days, until at the end of three weeks a new crop failing to appear, the electrolysis was discontinued. The crystals after being washed with dilute alcohol were found to melt at 189° (corrected). Repeated recrystallization from hot alcohol and benzene raised the melting-point to 191.2° (corrected). The crystals from benzene were well developed, colorless cubes. From 40 grams of acetylacetone only about 0.8 gram of pure crystals was obtained for analysis and further study.

Combustions with copper oxide gave the following figures:

I. 0.2041 gram substance gave 0.4528 gram CO_2 and 0.1298 gram H_2O.

II. 0.2094 gram substance gave 0.4614 gram CO_2 and 0.1326 gram H_2O.

III. 0.2088 gram substance gave 0.1329 gram H_2O.

	Calculated for $C_{10}H_{14}O_4$.	Found		
		I.	II.	III.
C	60.61	60.50	60.10	...
H	7.07	7.07	7.03	7.07
O	32.32	32.43	32.87	...
	100.00	100.00	100.00	

The material used for the combustions had previously been employed for molecular-weight determinations in benzene solution in the Beckmann freezing-point apparatus. Tetracetylethane is so sparingly soluble in benzene that only two observations of the lowering of the freezing-point could be made in one series. The molecular weight calculated for $C_{10}H_{14}O_4$ is 198; that found was 183 and 184.

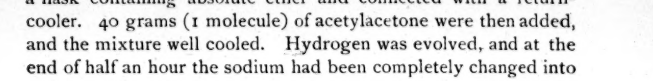
Like acetylacetone, tetracetylethane is a weak acid. It dissolves quickly and completely in solutions of alkaline hydroxides

and with some difficulty in alkaline carbonates. Stronger acids precipitate it from these alkaline solutions with its melting-point unchanged. A solution containing a drop of bromine in 5 cc. of chloroform was completely decolorized by 0.03 gram of tetracetylethane in less than two minutes without exposure to direct sunlight. A trace of hydrobromic acid was formed, but the rapidity of the decolorization suggested the idea that the action was mainly one of addition rather than of simple substitution.

The principal product of the electrolysis of acetylacetone which remained in the cells after the removal of the crystals was a reddish-colored syrup of aromatic odor but of most unpromising appearance, from which no definite compounds have yet been isolated.

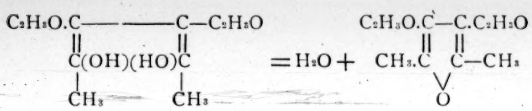
To prepare tetracetylethane in quantities sufficiently large to enable me to study its reactions more closely, I found myself forced to try the action of iodine on the sodium salt of acetyl acetone. 9.2 grams (1 atom) of fine sodium wire were placed in a flask containing absolute ether and connected with a return-cooler. 40 grams (1 molecule) of acetylacetone were then added, and the mixture well cooled. Hydrogen was evolved, and at the end of half an hour the sodium had been completely changed into white sodic acetylacetonate. 50.8 grams of iodine dissolved in dry ether were then slowly poured upon the mixture with constant shaking. When the reaction was ended the ether was distilled off and the sodium iodide dissolved out by water. The residue insoluble in water was tetracetylethane. It was purified by crystallization from boiling alcohol and melted at 191°. The yield was only 20 per cent. less than the theoretical.

A 1,4-diketonetetracetylethane should give several characteristic condensation-products. Thus, when its solution in glacial acetic acid is boiled with ammonium-acetate solution and then with dilute sulphuric acid and a pine-splinter—Knorr's pyrrol reaction—the splinter assumes a deep red color, showing that 1,4-dimethyl-2,3-diacetylpyrrol has been formed :



By an analogous condensation, in which one molecule of water is withdrawn from one molecule of tetracetylethane by concentrated

sulphuric acid, a quantitative yield of 1,4-dimethyl-2,3-diacetylfurfuran may be obtained:

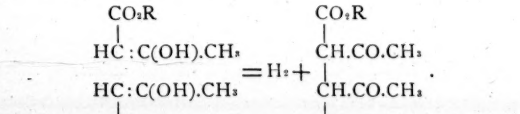


4 grams of tetracetylethane were dissolved in 7 cc. of cold concentrated sulphuric acid. Some heat was evolved. The solution, which was nearly colorless, was allowed to stand for ten minutes and then poured into about 50 cc. of cold water. The furfuran derivative soon crystallized out as a mass of pure white interlacing needles, weighing 3.5 grams. It dissolved readily in boiling water, and crystallized out, as the solution cooled, in long, silky needles having the appearance of glass-wool. When warm the body possesses a faint, agreeable aromatic odor. It melts at 63°. When analyzed it gave the following figures:

0.3273 gram substance gave 0.8008 gram CO_2 and 0.0214 gram H_2O.

	Calculated for $C_{16}H_{18}O_3$.	Found.
C	66.66	66.73
H	6.66	6.53
O	26.67	26.74

Electrolysis of Acetoacetic Ether (*Diacetylsuccinic Ether*).—As already stated, acetoacetic ether conducts the electric current slightly when dissolved in water; and in such a solution, if anywhere, one might expect that molecules containing the hydroxyl group and double bond would be present. On this assumption the following reaction might be anticipated:



It will be remembered that it is by the action of iodine upon the sodium salt of acetoacetic ether that diacetylsuccinic ether is usually prepared. This compound was not actually isolated from

the other products formed during the electrolysis of acetoacetic ether, but its presence among them was proved without difficulty.

25 grams of acetoacetic ether were dissolved in twice their weight of 50-per cent. alcohol and a current of one-seventh of an ampère passed through the solution for several days. This treatment of the acetoacetic ether gave rise to a thick oil, which, like diacetylsuccinic ether, upon being boiled, first with ammonium-acetate solution and then with dilute sulphuric acid, according to Knorr's method, gave a faint pyrrol reaction with a pine-splinter. Judging from the depth of color imparted to the splinter, the quantity of diacetylsuccinic ether escaping decomposition during the electrolysis must have been small. Later experiments made in this laboratory by Mr. Weems showed that when concentrated aqueous solutions of the sodium salt of acetoacetic ether are electrolyzed under slightly different conditions, the yield of diacetylsuccinic ether may become relatively large.

The type of electrosyntheses illustrated in the preceding pages is an interesting one and demands further study. It is the intention of the author to continue the investigation in the immediate future.

WORCESTER, MASS., *June*, 1893.

REVIEWS AND REPORTS.

LEHRBUCH DER ALLGEMEINEN CHEMIE. Von W. OSTWALD. IIte Auflage.—Band I (1163 pp., Mark 28); Band II, Erster Teil (1104 pp., Mark 34). Leipzig: W. Engelmann (1891–1893).

The appearance at this time of the first two volumes of the new edition of Ostwald's famous *Lehrbuch* will enhance not a little the rapidly growing interest which has been aroused by the wonderful development during the past decade of all phases of the theory of chemical processes. Since the completion of the first edition, five years ago, the discoveries in general physical chemistry—in particular in the Theory of Solutions, electrochemistry, and the subject of Chemical Equilibrium—have been so extended and of such fundamental character as to make it necessary not only

entirely to rewrite the work, but nearly to double its size. The historical method of treatment, which was the charm of the earlier edition, is preserved in the new; the clearness and exhaustiveness with which the subjects are presented render the book most fascinating reading.

The first volume, the *Stoechiometrie*, takes up the mass-relations of chemical compounds, with a critical recapitulation of the determinations of atomic weights, and then devotes six chapters to the laws of gases and thirteen to the properties of liquids. The Theory of Solutions is presented as a separate subject, and is followed by an extended discussion of the properties of solids, the volume closing with the Periodic Law and the Molecular Theory.

An unusual measure of interest is excited by the succeeding volume—on Chemical Energy—for the reason that here, for the first time in the history of chemical theory, the general doctrine of energy is exhaustively developed and applied. Thermochemistry, electrochemistry and photochemistry are regarded as treating the transformations between chemical energy, on the one hand, and heat, electricity and radiant energy on the other. The substantiality of energy, its forms, its capacity- and intensity-factors, and the laws of its transformations, are brought out and emphasized; the significance of the intensity-factor of chemical energy—the chemical potential—in the theory of chemical equilibrium is made apparent; the history of the energy doctrine is outlined.

That thermodynamics is a special phase of the energy doctrine clearly appears from the chapters on thermochemistry. Here, in addition to the theory, are collected from the literature all reliable thermochemical data—a rather remarkable task in itself. The "book" on electrochemistry is a brilliant piece of work. The historical development—from Grotthuss to the free-ion theory; from Hittorf and Kohlrausch to Arrhenius and Ostwald—is most interesting, and is followed by complete discussion of the magnificent results in the theory of concentration-currents and of the voltaic cell, the whole treated from one point of view: as necessary deductions from the laws of energy. Many new conclusions are drawn and confirmed by experiment; the whole reads like an original paper and is inspiring in the extreme. The volume is concluded by a similar presentation of the facts and theories of photochemistry.

The final volume (Band II, zweiter Teil), which is to appear later this year, will treat of Chemical Equilibrium, the Law of Mass-action and the applications of thermodynamics—in short, all that is known as the *Verwandschaftslehre*. Its coming will be awaited with no little interest. J. E. T.